cancer treatment reports

Proceedings of the 6th New Drug Seminar: DTIC

Volume 60
Number 2
February 1976
Pages 123-214

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

cancer treatment reports

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Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402. The yearly subscription rate is $38.35 domestic, with an additional $9.60 for foreign mailing. The prices vary for individual issues. Payment is required in advance; check or money order should be made payable to the Superintendent of Documents. Please specify List ID "CAN" when requesting a subscription.

Sixth New Drug Seminar on DTIC*

Division of Cancer Treatment
National Cancer Institute

Editor: Stephen K. Carter

April 25, 1975
Bethesda, Maryland

*DTIC is now commercially available under the trade name of DTIC-Dome.

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

History and Development of DTIC (NSC-45388)[1]

Saul A. Schepartz[2,*]

The National Cancer Institute's Drug Research and Development Program becomes involved with drugs in a variety of ways and at various stages of development. In some cases, the NCI may not become involved until the drug is already in clinical trial in this or another country. In other cases, preliminary data may have been obtained by the investigator who discovered the drug and the NCI may become involved heavily in the preclinical development and eventual clinical evaluation. In still other cases, the NCI may be provided with a new compound of unknown biologic activity to evaluate in its screening systems. The majority of such compounds are submitted without charge by chemists in the chemical and pharmaceutic industry or in academic and other institutes throughout the world. A relatively small proportion of the total compounds submitted to the NCI come from within its own contract-supported research programs. DTIC is one of these rare compounds. It serves as a good example of the collaboration of the NCI with its contractors in academic, industrial, and research institutes as well as with clinical and other investigators throughout the world. The details relating to the collaborative development of this drug will become clear in the papers presented in this issue. It might be of interest, however, to trace very briefly the chronology of the development of DTIC from its original synthesis to the present. This chronology is summarized in table 1.

The compound was first synthesized in 1959 by investigators at Southern Research Institute under an NCI contract. It was tested in the NCI in vivo screen shortly after that and in the course of testing, activity was observed in L1210 leukemia. Over the next 3 years, a great deal of additional biologic testing was carried out to establish fully the parameters of activity of this new compound. Finally, in April 1964 a decision was made to develop DTIC toward preclinical toxicology testing and clinical trial. This further development involved increasing the production of the drug so that it could be produced on a scale sufficient for toxicologic and clinical evaluation, developing a suitable formulation so that the drug could be administered appropriately to patients, and finally, designing the large animal toxicology studies. Toxicology studies tested the drug by both the oral and iv routes. An Investigational New Drug Application (IND) was filed in March 1966 for oral use and a supplement was filed in September 1966 for iv use. Over the next several years, extensive clinical studies were carried out, finally leading the NCI to the conclusion that DTIC was indeed an active drug and worthy of being put on the market. Early in 1973 efforts were initiated to develop a contract for processing a New Drug Application (NDA) which would eventually lead to the marketing of this drug. A contract to carry out this activity was signed in September 1973, the NDA was filed in February 1974, and 1 year later the so-called

TABLE 1.—Chronology of DTIC development

Date	
10/59	First sample synthesized by Southern Research Institute
2/60	Submitted to NCI screen
3/61	Activity in L1210 leukemia first observed
4/64	Accepted for development toward clinical trial
12/64-3/66	Formulation development
3/66-9/66	First large production batches prepared
11/64	Oral toxicology studies started
7/65	Oral toxicology studies completed
3/66	IND application filed
5/65	Iv toxicology studies started
4/66	Iv toxicology studies completed
9/66	IND amendment filed
1/73	RFP issued for marketing
9/73	Marketing contract signed
2/74	NDA filed
2/75	"Approvable" letter received

[1]DTIC: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

[2]Drug Research and Development Program (DR&DP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

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approvable letter was received, thus signifying a final stage in the approval process. Final approval and marketing should be occurring very soon.

It may appear frustrating to view the overall sequence of events and what appears to be a rather long time from the initial synthesis of the drug to the present. It is indeed long, but as mentioned briefly above, a great many events must occur in the course of this development. The majority of drugs, of course, never reach this point. It is of value for the scientific and lay public to be aware of the time that is frequently and usually required for moving a new drug from its original synthesis to the point where it can be placed on the market. Obviously, we are attempting to speed up this process, and we hope that in the future new drugs will be arriving at the marketplace more rapidly. It will continue to be a long hard road, however, from the initial concept to the actual use of any new active drug.

Experimental Studies at Southern Research Institute With DTIC (NSC-45388)[1]

John A. Montgomery[2,*]

SUMMARY

Several years ago the diazotization of 5-aminoimidazole-4-carboxamide was found to give an internal diazonium salt, 5-diazoimidazole-4-carboxamide, which demonstrated marginal activity against some murine neoplasms. In an effort to improve this activity and develop a clinically useful agent, this compound was converted to a number of derivatives including DTIC, an agent that showed encouraging activity against a variety of rodent neoplasms. Studies carried out at Southern Research Institute on the chemistry, metabolism, and biologic activity of DTIC and its congeners are discussed briefly.

[Cancer Treat Rep 60:125-134, 1976]

Some time ago chemists at Southern Research Institute embarked upon a program designed to uncover new inhibitors of the biosynthesis of nucleic acids. At the time, most of the known inhibitors of this pathway were analogs of the naturally occurring purines or pyrimidines (1). There appeared to be a good possibility of developing useful inhibitors that were analogs of 5-aminoimidazole-4-carboxamide (AIC) and its phosphoribosyl derivative (fig 1). Another project undertaken at that time was the synthesis of ring analogs of purines and their nucleosides among which were new 2-azapurines.

The synthesis of 2-azahypoxanthine by Woolley and Shaw (2), as depicted at the top of figure 2, had appeared in the literature. Treatment of AIC with nitrous acid gave, according to those authors, 2-azahypoxanthine. When this reaction was carried out at Southern Research Institute, a different product was obtained—a compound that, because of its strong absorption in the 2200 region of its infrared spectrum, was obviously not 2-azahypoxanthine. Elemental analyses with the infrared spectral data and a positive Bratton-Marshall test (3) proved that the material was an internal diazonium salt—5-diazoimidazole-4-carboxamide (diazoIC) (fig 2) (4). Furthermore, on standing in solution (either acid or base) the diazoimidazole was converted into 2-azahypoxanthine.

There is a striking similarity between the structure of the diazoimidazole and those of the anticancer antibiotics azaserine (5) and DON (6), compounds that were known to inhibit an early step in the de novo purine nucleotide biosynthetic pathway (7,8) (fig 3). These compounds block the conversion of phosphoribosyl-formylglycinamide to phosphoribosyl-formylglycinamidine by alkylation of a thiol residue at the active site of phosphoribosyl-formylglycinamidine synthetase, the enzyme that catalyzes this transformation (9). It appeared quite possible that the diazoimidazole might interfere irreversibly with a later step on the pathway beyond the formation of the imidazole nucleus (fig 3). Because of this, the compound was tested and found to be cytotoxic to H.Ep. No. 2 cells in culture and was then screened for activity against several rodent neoplasms (4). It proved to have some activity against Ehrlich ascites carcinoma and Walker adenocarcinoma 256, slight activity against Sarcoma 180, and little or no activity against Adenocarcinoma 755 and L1210 leukemia (table 1).

Since the compound did, in fact, show biologic activity, even though it appeared that the activity was not great enough to make the compound of any potential value for the treatment of human cancer, ways were sought to convert the diazoimidazole into derivatives that might have greater selectivity for cancer cells and, therefore, greater potential clinical utility. Among these derivatives were the reaction products of the diazoimidazole

[1]Supported by contract N01-CM-43762 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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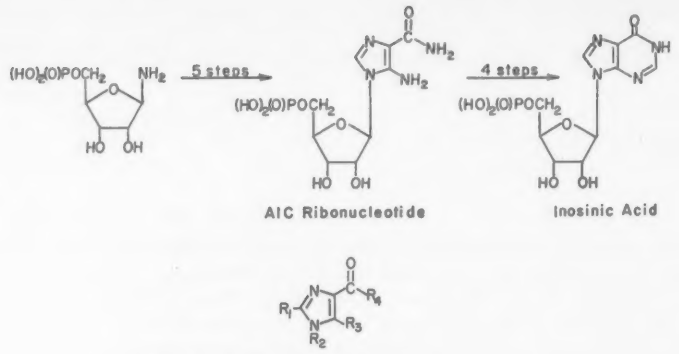

FIGURE 1.—De novo biosynthesis of purine nucleotides.

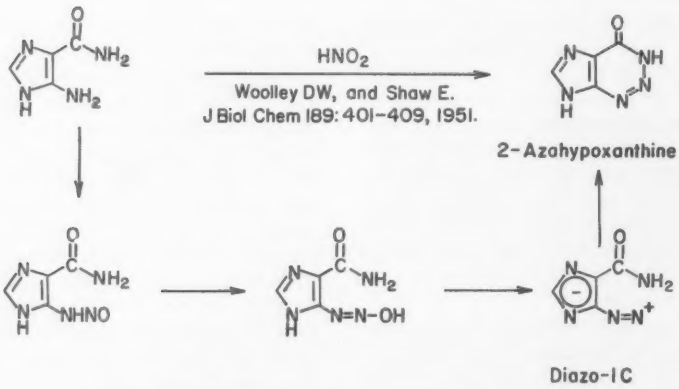

FIGURE 2.—Synthesis of 2-azahypoxanthine.

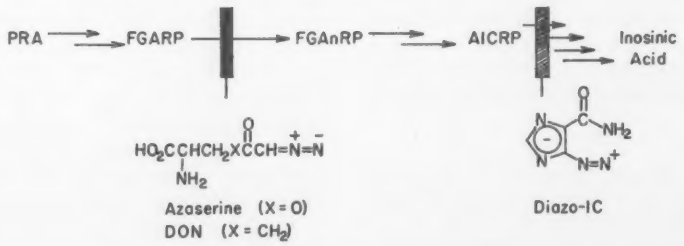

FIGURE 3.—Possible inhibition of de novo purine nucleotide biosynthesis by diazo-IC.

TABLE 1.—Diazo-IC vs experimental rodent tumors

Dose (mg/kg/dose, ip)	Schedule	Route	Tumor	Host weight change (g), T/C	Mortality (Day 5)	Average tumor weight (mg) or average lifespan (days)		TWI* or ILS† (%)
						Treated	Control	
15	qd Days 1-11	ip	Ehrlich ascites carcinoma	—	6/10	—	—	—
10				+0.7/+4.3	2/10	4.44	2.48	46
5				+1.3/+4.3	2/9	0.43	2.48	17
10				+0.2/+10.8	1/10	0.89	6.14	14
5				+4.6/+10.8	1/10	1.11	6.14	18
20	qd Days 1-7	ip	Sarcoma 180	—	6/6	—	—	—
10				—	3/5	—	—	—
5				-0.3/+0.9	2/6	555	996	55
2.5				+1.8/+0.9	1/6	532	996	53
1.3				+1.8/+0.9	0/6	609	996	61
5	qd Days 1-11	ip	Adenocarcinoma 755	—	7/10	—	—	—
2.5				—	5/10	—	—	—
1.3				+1.8/+1.3	2/10	659	1076	61
10	qd Day 1 to death	ip	L1210 leukemia	—	7/9	—	—	—
7.5				-2.0/+0.5	0/10	8.7	9.3	0
5				-1.6/+0.5	0/10	9.7	9.3	4
2.5				-1.0/+0.5	0/10	9.4	9.3	1
20	qd Days 1-11	ip	Walker adenocarcinoma 256	+36.7/+66.2	1/6	251	1477	17
20				—	5/6	—	—	—
10				+33.6/+59.2	1/6	345	1646	21
5				+51.2/+59.2	0/6	1152	3032	38

*TWI = tumor weight index.
†ILS = increase in lifespan.

TABLE 2.—Activity of DTIC vs L1210 leukemia (ip treatment, single dose)

	Dosage		Median lifespan (days)		% ILS	Log kill
	mg/kg	× LD10	Treated	Control		
10^5 Cells, ip	720	2.0	6.0	8.0	toxic	
	480	1.2	15.5	8.0	93	≥4
	320	0.8	14.5	8.0	81	≥4
	300	0.75	13.0	8.0	62	≥3
	200	0.50	13.0	9.0	44	≥3
	150	0.40	11.0	8.0	37	≥1
	140	0.34	11.5	9.0	27	≥2
	90	0.23	11.5	9.0	27	≥2
	75	0.20	9.5	9.5	18	<1
	38	0.10	9.0	8.0	12	none
10^4 Cells, ic	720	2.0	4.5	9.0	toxic	
	480	1.2	8.0	9.0	0	none
	320	0.8	9.0	9.0	0	none

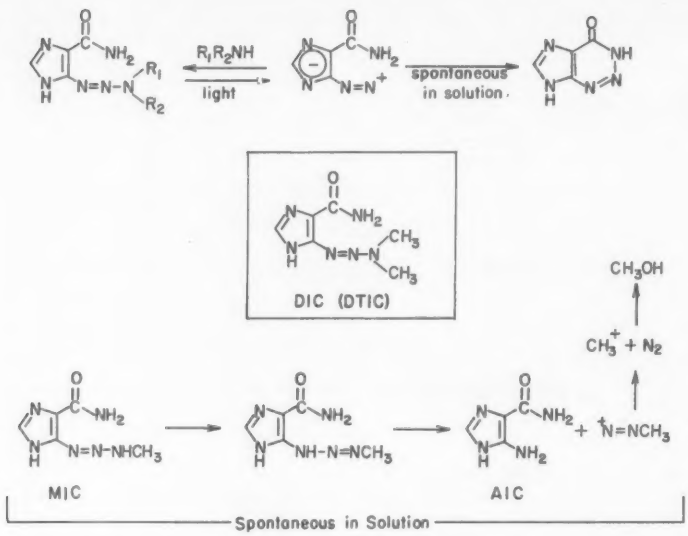

FIGURE 4.—5-Triazenoimidazole-4-carboxamides.

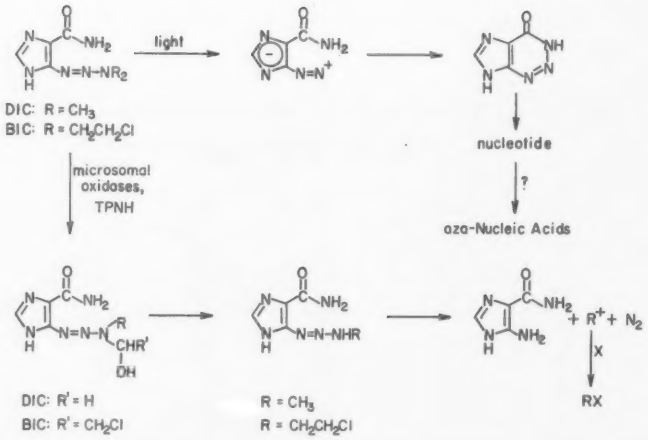

FIGURE 5.—Metabolic activation of the 5-dialkyltriazenoimidazole-4-carboxamides.

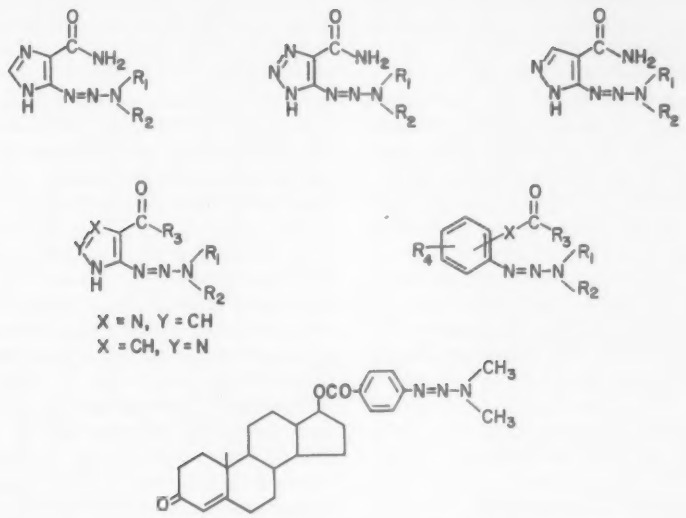

FIGURE 6.—Structural variations of DTIC.

TABLE 3.—Dose regimen study: DTIC vs L1210 leukemia

Optimal dose (mg/kg/dose)	Route	Schedule	Median lifespan (days)		% ILS
			Treated	Control	
100	ip	qd Days 1-30	17.7	9.0	96
100			17.2	10.0	72
100			15.0	8.9	68
120	ip	qod Days 1-19	12.8	8.9	44
50	ip	2 injections daily Days 1-30	12.0	8.2	46
50			11.7	9.0	30
150	ip	q3d Days 1-30	15.5	8.5	82
150			12.5	8.2	52
175	ip	q7d Days 1-30	13.0	8.2	58
175			13.2	8.5	55
480	ip	Day 1 only	15.5	8.0	93
500	ip	Day 5 only	11.8	8.5	38
100	sc	qd Days 1-30	14.2	9.0	57
100	im	qd Days 1-30	14.5	9.0	61
100	iv	qd Days 1-6	13.2	9.0	46
200	oral	qd Days 1-30	15.7	8.2	91
100	oral	qd Days 1-30	11.3	8.2	37

with amines—the 5-triazenoimidazole-4-carboxamides (fig 4) (10). Stability studies showed that ultraviolet light, or just sunlight, was capable of converting these triazenes back to the diazoimidazole and on to 2-azahypoxanthine (10). Although this light instability presented a problem, it was not a serious one. However, because of it, these compounds were protected from light during screening and fresh solutions were prepared daily. The first compound of this type to be synthesized was the dibutyl triazene [$R_1 = R_2 = Me(CH_2)_3-$], which, as it turned out, was much less active than the dimethyl triazene DTIC ($R_1 = R_2 = Me-$) (11).

Another slightly different type of derivative was prepared shortly afterward; the amine that was allowed to react with the diazoimidazole was a primary amine and, therefore, the linear triazene formed carried only one alkyl group (12). The monomethyl analog (MIC) of DTIC is pictured at the bottom of figure 4. These monoalkyltriazenes were found to be quite different chemically from the dialkyltriazenes. In solution, the double bond of the original triazene rearranges as depicted, and then dissociation takes place between two of the nitrogens to regenerate AIC, from which the diazoimidazole was originally prepared, with the formation of an alkyldiazonium cation, which further decomposes to give an alkyl cation and nitrogen (12). The alkyl cations, of course, are highly reactive species with a short half-life, and in the presence of water alone they react with water as a nucleophile to form alcohols primarily (13). In spite of the difference in chemical behavior, MIC was about as active as DTIC against animal tumor systems.

The consequences of this chemical and biologic activity were shown to be very important later when the metabolism of DTIC was investigated. It was shown by Skibba et al (14) that DTIC is oxidatively demethylated in vivo and in vitro by liver microsomes to give, presumably, the monomethyl compound, MIC, which then undergoes the kind of decomposition depicted in figure 4 to give AIC (14,15) and a methyl cation. In living cells, however, this highly reactive species then reacts with many nucleophiles including the nucleic acids (fig 5). The methylation of DNA is probably ultimately responsible for the activity of DTIC. Nevertheless, when DTIC is given to animals, several potentially cytotoxic compounds can be evolved: the methyl cation, the diazoimidazole, and 2-azahypoxanthine, which is converted by cells to its nucleotide and perhaps incorporated into nucleic acids (16). Currently most investigators agree, however, that it is the oxidative demethylation which activates DTIC by converting it to the monomethyltriazenoimidazole.

Recently at Southern Research Institute, Hill has found that microsomal oxidases are equally capable of oxidizing the chloroethyl group and that, in fact, when BIC (17,18) is allowed to incubate with a microsomal oxidase preparation in the presence of TPNH, AIC is formed (19). Therefore, one chloroethyl group must be removed to give chloroacetaldehyde and 5-[3-(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide, a compound as active against L1210 as BIC but at one tenth the dose (20). That this compound is a source of chloroethyl cations was shown by the fact that its decomposition in water gave a high yield of 2-chloroethanol (20). Thus activation would appear to play a vital role in the antileukemic activity of this analog also.

A number of structural variations of DTIC have been prepared in the hope of obtaining an even better compound. Most of these variations are summarized in figure 6. The diazoimidazole was allowed to react with a number of monoamines and dialkylamines (10,12,21,22); the triazole analog of DTIC and many derivatives have been made (23); the pyrazole analog of DTIC and many variations of the terminal amino function of this congener have been prepared (22,24). Also synthesized are a number of ester analogs containing either the imidazole, triazole, or pyrazole ring (25,26), as indicated in the middle of figure 6, and quite a number of variants in which the imidazole ring is replaced by benzene or substituted benzene moieties (27,28). Some of these latter structures fairly closely resemble DTIC itself (28), and some of them are more closely related to drugs such as melphalan or chlorambucil (27). At the bottom of figure 6 is depicted one of a number of modified steroids that contains a dimethylphenyltriazene grouping.

Many of the compounds shown in this figure do, in fact, show activity in the L1210 system, but none of them are significantly better than DTIC, with the exception of the compounds already mentioned, ie, BIC (17,18) and the monochloroethyl derivative (20). Both of these compounds are significantly better than DTIC in the L1210 system, giving at the appropriate dose level and on the appropriate schedule large numbers of cures of leukemic animals (17,20). Unfortunately, the clinical results with BIC have not been encouraging, perhaps because of formulation difficulties arising from the facile transformation of BIC in aqueous media into an inactive triazoline (17,18).

Biologic Evaluation

Some of the early screening data obtained with DTIC against L1210 leukemia are given in table 2.

It was these data plus the activity against Sarcoma 180 and Adenocarcinoma 755 (11) that created the interest that led to the development of this compound. As can be seen from this table, at nontoxic doses against L1210 leukemia injected ip (10^5 cells), > 4 logs of cells can be killed, giving significant increases in lifespan and a fairly good therapeutic index (about 5). The compound is not active against L1210 leukemia if the cells are injected ic into mice, as can be seen from the data at the bottom of table 2.

Dose-regimen studies have been carried out with ip administration (table 3). Quite a variety of scheduling was evaluated: qd Days 1-30, every other day, twice daily, every third day, every seventh day, Day 1 only, and Day 5 only. In general, there is not very much effect at the optimal dose by the variation in schedule with the exception of the Day 5 only schedule. In that case the leukemia is advanced before initiation of treatment and so the increase in lifespan is significantly less. Table 3 also shows results obtained with the drug administered by other routes, sc, im, iv, and orally, and again it is plain that the activity is not too different whatever the route of administration.

Table 4 shows the activity of DTIC against three lines of L1210 leukemia that are resistant to other agents (methotrexate, 6-mercaptopurine, and 6-thioguanine). DTIC is just as active against these resistant cell lines as it is against the parent line.

The activity of DTIC against other mouse leukemias is shown in table 5; except for the last leukemia listed, P1798, DTIC is quite active, giving a number of cures against the P815/FU leukemia and against the L4946 leukemia.

DTIC was found to be active against Sarcoma 180 and Adenocarcinoma 755 in addition to its activity against L1210 leukemia (table 6). It is more active against Adenocarcinoma 755 than against Sarcoma 180, giving some cures. It is quite active against the P1798 lymphosarcoma, curing ten out of ten animals at the levels indicated, and it is slightly active against Walker adenocarcinoma 256, but only with large host weight differences (T/C).

Table 7 shows the results of combinations of single agents with DTIC against L1210 leukemia. The first compound listed, phenobarbital, might be expected to enhance the oxidative demethylation of DTIC, and the second compound, SKF-525-A (29), might be expected to inhibit the oxidative demethylation; however, neither one seems to have much effect on DTIC's activity against L1210. None of the antimetabolites tested (8-azaguanine, 6-thioguanine, 5-FUdR, and hydroxyurea) potentiate with

TABLE 4.—Activity of DTIC vs resistant lines of L1210 leukemia (10^6 cells ip)

Cell line	Dose (mg/kg/dose, ip)	Schedule	Median lifespan (days)		% ILS
			Treated	Control	
L1210/MTX	125	qd Days 1-30	12.5	7.0	78
	50		12.5	7.0	78
	33		12.5	7.0	78
	20		9.0	7.0	28
	150	qd Days 1-15	9.0	8.0	12
	125		10.0	8.0	25
	125		14.0	7.0	100
	100		11.0	8.0	37
	87		14.0	7.0	100
	62		13.5	7.0	92
	42		9.0	7.0	28
L1210/MP	125	qd Days 1-30	12.0	7.0	71
	125	qd Days 1-15	11.0	7.0	57
	100		11.5	13.0	0
	75		16.0	13.0	23
L1210/TG	125	qd Days 1-15	14.5	10.0	45
	100		13.0	10.0	30
	100		15.0	10.0	50
	75		11.5	10.0	15
	75		18.5	10.0	85

TABLE 5.—Activity of DTIC vs other mouse leukemias

Cell line	Optimal dose (mg/kg/dose, ip)	Schedule	Median lifespan (days) Treated	Control	% ILS
P815	75	qd Days 1-15	>24.1	14.3	>68 (1/10)
P815/FU	100		>28.6	14.7	>95 (5/10)
L5178Y	75		12.2	9.2	32
L4946	75		>21.8	11.4	>91 (2/10)
L4946/Az	125		19.4	11.4	63
P1798	inactive				

TABLE 6.—DTIC vs solid tumors

Tumor	Optimal dose (mg/kg/dose, ip)	Schedule	Host weight change (g), T/C	Average tumor weight, T/C	% of control
Sarcoma 180	100	qd Days 1-7	-2.2/-2.2	323/1101	29
	100		1.6/+1.2	340/996	34
	100		-2.0/+3.6	323/1348	23
	100		-2.0/+1.8	447/1818	24
	100		-0.8/+0.3	427/1048	50
Adenocarcinoma 755	125	qd Days 1-11	-2.4/+2.2	139/1164	11 (1/10)
	125		-3.7/+4.5	72/1933	3 (1/10)
	125		-1.8/+2.4	239/820	29
	125		-3.1/+0.1	213/1047	20
	125		-3.8/+0.2	114/782	14
	125		-2.4/+2.8	587/1316	44
P1798 lymphosarcoma	100	qd Days 1-11	-3.4/+1.6	0/490	0 (10/10)
	75		-4.1/+1.6	0/490	0 (10/10)
Walker adenocarcinoma 256	500	qd Days 1-15	-28/+57	2.6/5.4	48 (1/6)

TABLE 7.—Combination chemotherapy: DTIC + single agents vs L1210 leukemia

Agent	Potentiation
Phenobarbital	–
SKF-525-A	–
8-Azaguanine	–
6-Thioguanine	–
5-FUdR	–
Hydroxyurea	–
Melphalan	+
Cyclophosphamide	+, –
BCNU	–
CCNU	–(BIC +)
Adriamycin	+

TABLE 8.—Combination chemotherapy: DTIC + adriamycin vs solid tumors

Tumor	% ILS Adriamycin	DTIC	Combination
B16 melanoma	23	21	138
C3H breast carcinoma	58	54	>170
Lewis lung carcinoma	14	0	31

DTIC. However, melphalan, the phenylalanine mustard, does potentiate with DTIC and this potentiation has been demonstrated several times. Cyclophosphamide showed some potentiation, but this result was not readily reproducible. There is no potentiation of DTIC with the nitrosoureas and, in fact, what is observed is greater than additive toxicity (30). This is in contrast to the results obtained with BIC, which is highly potentiating with CCNU (30). The last combination, adriamycin and DTIC, was first used in the clinic, and the animal data simply support what was observed in man, that is, that adriamycin and DTIC are potentiating, not only in the L1210 leukemia system, but also in solid tumors in rodents (table 8). The combination

of DTIC and adriamycin against the B16 melanoma, the C3H breast carcinoma, and the Lewis lung carcinoma gives better results, in terms of increase in lifespan, than either drug alone (31). All of these tests were run at the LD10 of either the individual drug or of the combination.

This brief survey covers most of the studies carried out with DTIC and its congeners at Southern Research Institute.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

8-Azaguanine: NSC-749; CAS reg. No. 529-99-7; 7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one, 5-amino-1,6-dihydro-

2-Azahypoxanthine: NSC-22419; CAS reg. No. 7151-03-3; 7*H*-imidazo[4,5-*d*]-*v*-triazin-4-ol, hydrate

Azaserine: NSC-742; CAS reg. No. 115-02-6; serine, diazoacetate (ester), L-

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

BIC: NSC-82196; CAS reg. No. 5034-77-5; imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

5-[3-(2-Chloroethyl)-1-triazeno]imidazole-4-carboxamide: NSC-157949; CAS reg. No. 28177-14-2

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Diazo-IC: NSC-22420; CAS reg. No. 28749-61-3; imidazole-4-carboxamide, 5-diazo

5-(3,3-Dibutyl-1-triazeno)imidazole-4-carboxamide: NSC-45396; CAS reg. No. 4342-09-0

DON: NSC-7365; norleucine, 6-diazo-5-oxo-, L-

DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

5-FUdR: NSC-27640; uridine, 5-fluoro-2′-deoxy-

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

MIC: NSC-407347; CAS reg. No. 3413-72-7; imidazole-4-carboxamide, 5-(3-methyl-1-triazeno)-

SKF-525-A: NSC-39690; CAS reg. No. 62-68-0; valeric acid, 2,2-diphenyl-, 2-(diethylamino)ethyl ester, hydrochloride

6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-

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Antitumor Activity of DTIC (NSC-45388) in Animals[1]

John M. Venditti[2,*]

DTIC was synthesized at the Southern Research Institute (1) as one of a number of triazenoimidazoles and was initially screened for antitumor activity in rodents at that institute (2). Clinical or preclinical findings for DTIC have been reported in over 150 published studies. The purpose of this report is to summarize pertinent information from selected animal antitumor tests conducted in the NCI contract program. In general, the experimental methods conformed to DR&DP protocols for antitumor screening (3). Unless otherwise noted, DTIC was suspended in 0.5% carboxymethylcellulose (CMC) in physiologic saline with or without Tween 80 or in 0.3% hydroxypropylcellulose in saline. The route of administration and treatment schedule are noted in the tables of results.

Table 1 summarizes the influence of route of administration and treatment schedule on the lethality of DTIC to BDF_1 mice. Calculated LD10s from pooled normal mouse testing are relatively high compared with most antitumor drugs. The oral LD10 for single-dose treatment was approximately 1½ times the sc LD10 and about twice the ip LD10. However, within each of the multiple-treatment schedules shown, the route of administration had little influence on the LD10. Also, comparison of the various multiple-treatment schedules suggests that for ip treatment the total LD10 (over a 9-day treatment period) was moderately influenced by the interval between treatments. Thus, for ip treatment the total LD10s were 873, 786, and 408 mg/kg when given daily for 9 days; once on Days 1, 5, and 9; or every 3 hours on Days 1, 5, and 9 respectively. In contrast, when the drug was given orally, the total LD10s were 1026, 609, and 384 mg/kg when given daily, once every fourth day, or every 3 hours on every fourth day respectively.

DTIC was developed to clinical trial on the basis of its activity against ip implanted leukemia L1210. In the experiments summarized in table 2, a range of dosage levels was given for each route and treatment schedule to leukemic mice and to parallel groups of normal mice. The table shows the optimal dose for each route and schedule, ie, the dose that produced the maximum percentage increase in the lifespan (ILS) of leukemic mice. Where the optimal dose exceeded the estimated LD10 for normal mice treated in the same experiment, the results are enclosed in parentheses. The therapeutic efficacy of DTIC against L1210 was moderate, but highly significant. At nonlethal doses, the maximum ILS achieved ranged from 43% (treatment every 3 hours, every fourth day) to 67% (treatment daily, Days 1-9). Thus, DTIC was active on each of the schedules used and was not markedly schedule dependent. Typical anti-L1210 activity was retained when the drug was given sc or orally (table 2). Table 3 summarizes the activity of DTIC against a number of drug-resistant variants of L1210 and against other mouse leukemias. Activity was observed against L1210 sublines which were developed for resistance to methotrexate, 6-mercaptopurine, and 6-thioguanine. DTIC treatment resulted in long-term survivors among mice bearing leukemia P815 or a 5-fluorouracil (5-FU)-resistant variant of P815. The activity of DTIC against leukemia P388 was comparable to its activity against L1210 (table 3). Thus, DTIC appears to exert selective toxicity against a number of rapidly proliferating tumors by mechanisms different from those implicated in the antitumor activity of widely used antitumor antimetabolites.

The activity of DTIC against rodent solid tumors varied widely (table 4). The results shown were observed at optimal dosage levels or at the LD10 of DTIC. Inhibition of growth of the local tumor at the site of implantation was observed against mouse Sarcoma 180, Carcinoma 755, and lymphosarcoma P1798. DTIC used alone was essentially inactive or minimally effective against B16 melanoma, Lewis lung carcinoma, and Ridgway osteogenic sarcoma in mice, and against Walker

[1]The work reported in this paper was supported by contracts NO1-CM-43756 (Southern Research Institute, Birmingham, Ala), NO1-CM-33727 (Arthur D. Little, Inc, Cambridge, Mass), NO1-CM-33728 (Microbiological Associates, Inc, Bethesda, Md), NO1-CM-12182 (Battelle Memorial Institute, Columbus, Ohio), and NO1-CM-23704 (Hazleton Laboratories, Vienna, Va) from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

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TABLE 1.—DTIC toxicity in nontumor-bearing BDF_1 mice*

Treatment				
Route of administration	Schedule	LD10	LD50 (mg/kg/injection)	LD90
Ip	Single dose	626	859	1177
Sc	Single dose	820	> 1200	> 1200
Oral	Single dose	> 1200	> 1200	> 1200
Ip	q4d × 3	262	333	406
Oral	q4d × 3	203	351	688
Ip	qd × 9	97	150	266
Sc	qd × 9	104	180	312
Oral	qd × 9	114	—	—
Ip	q3h × 8, q4d × 3	17	34	69
Oral	q3h × 8, q4d × 3	16	50	—

*Data of Schabel et al, Southern Research Institute. DTIC suspended in CMC or CMC + Tween 80.

256 carcinoma in rats. The activity against an early generation transplant of C3H mammary carcinoma (73% ILS) is noteworthy.

The soluble clinical formulation of DTIC containing mannitol and citric acid retained its anti-L1210 activity. In the experiment summarized in table 5, the clinical formulation (dissolved in water) produced a 62% ILS at an optimal dose of 125 mg/kg of DTIC compared with a 50% ILS at an optimal dose of 100 mg/kg for bulk drug suspended in CMC.

DTIC failed to produce its typical anti-L1210 effect when the tumor was implanted ic (table 6). However, conclusions regarding the ability of the

TABLE 2.—Activity of DTIC against mouse leukemia L1210*

Treatment			
Schedule	Route of administration	Optimal dose (mg/kg/dose)	ILS (%)
Day 1 only	Ip	600,300	51,56
	Sc	(1200),600	(74),50
	Oral	1200,1200	65,50
qd, Days 1-9	Ip	100,100	67,62
	Sc	(200),100	(98),62
	Oral	(200),100	(96),62
q4d, Days 1,5,9	Ip	(300),150	(54),49
	Oral	(1200),150	(96),40
q3h,q4d, Days 1,5,9	Ip	(60),30	(72),43
	Oral	(120),30	(73),62

*DR&DP screening data. Summary of 2 experiments. 10^5 L1210 cells were injected ip. Nos. in parentheses indicate that the optimal antileukemic dose exceeded the LD10 for nontumor-bearing mice treated in parallel.

TABLE 3.—Activity of DTIC against mouse leukemias including drug-resistant variants

Ip tumor (10^6 cells)	Optimal dose for ip treatment, qd, Days 1-15 (mg/kg/day)	ILS (%)	30-Day survivors/ total No. treated
L1210/MTX*	87	100	0/10
L1210/6-MP*	125	57	0/10
L1210/6-TG*	75	85	0/10
P815*	100	> 45	2/10
P815/5-FU*	100	> 95	5/10
P388†	100	50	0/12

*Data from Southern Research Institute.
†Data from Arthur D. Little, Inc. Treatment given ip, qd, Days 1-10.

TABLE 4.—Activity of DTIC against rodent solid tumors

Tumor (implant site)	Ip treatment schedule	Tumor weight inhibition (%)	ILS (%)
B16 melanoma (sc)*	qd, Days 1-9	—	43
B16 melanoma (ip)*	qd, Days 1-9	—	29
Lewis lung carcinoma (sc)†	qd, Days 2-9	44	0
Sarcoma 180 (sc)†	qd, Days 1-7	77	—
Carcinoma 755 (sc)†	qd, Days 1-11	97	—
Lymphosarcoma P1798 (sc)†	qd, Days 1-11	100	—
Ridgway osteogenic sarcoma (sc)†	qd, Days 12-18	17	0
C3H mammary carcinoma (sc)†	qd, Days 2-9	—	73
Walker 256 carcinoma (sc)†	qd, Days 1-5	52	—

*DR&DP screening data.
†Data from Southern Research Institute.

drug to cross the mouse blood-brain barrier from this observation should be made cautiously. Geran et al (4) have reported DTIC to be effective against ic implants of a transplantable mouse ependymoblastoma.

TABLE 5.—Activity of clinical formulation of DTIC against ip L1210 (10^5 cells)

Drug formulation	Optimal dose, DTIC equivalents, for ip treatment, qd, Days 1-5 (mg/kg/day)	ILS (%)
Clinical* (dissolved in water)	125	62
Placebo†	(62.5-500)†	0
Bulk suspended in CMC	100	50

*200 mg DTIC, 50 mg mannitol, 200 mg citric acid/vial.
†50 mg mannitol, 200 mg citric acid/vial.

Kline et al (5) have demonstrated that the activity of DTIC against early L1210 tumor carried over to the situation in which sc treatment of mice with sc implanted tumor was delayed until the disease was systemic. In the same studies the therapeutic potentiation of DTIC plus cytosine arabinoside against advanced L1210 was shown. Moreover, methotrexate and BCNU given alone or combined after therapy with DTIC plus cytosine arabinoside provided ILSs that exceeded those observed in mice in which DTIC plus cytosine arabinoside treatment was continued. Bioassay studies showed the rapid onset of resistance to cytosine arabinoside treatment in animals that continued to respond to DTIC or methotrexate. The study suggested that the developing resistance to cytosine arabinoside coupled with the moderate activity of DTIC against the tumor limited the usefulness of the combina-

TABLE 6.—Lack of DTIC activity against ic implanted L1210*

Implant site (cells)	Optimal dose for ip treatment given on Day 1 only (mg/kg)	Increase in median survival time (%)	30-Day survivors/ total No. treated
Ip (10^5)	317	81	0/6
Ic (10^4)	317	0	0/6

*Data from Southern Research Institute.

tion; this limitation could be overcome, to some extent at least, by sequential treatment with an alternative synergistic drug combination (5). The therapeutic potentiation of DTIC plus cyclophosphamide, 5-FU, or adriamycin against L1210 was previously reported from the DR&DP (6,7). The combination of DTIC plus adriamycin is of particular interest since Gottlieb et al (8,9) have reported that these drugs, each of which was effective when used alone, appeared to be significantly superior when used in combination against a wide variety of metastatic sarcomas in man. Schabel (10) and Griswold et al (11) at the Southern Research Institute demonstrated the therapeutic potentiation of DTIC plus adriamycin against a variety of animal solid tumors including sc implanted B16, Lewis lung carcinoma, and early passaged C3H mammary carcinoma, as well as against leukemia L1210. It should be remembered that DTIC when used alone showed only minimal activity against B16 melanoma and Lewis lung carcinoma (table 4). Examples of the effectiveness of the combination of DTIC plus adriamycin against L1210, B16 melanoma, and C3H mammary carcinoma are shown in tables 7-9. For each experiment, the results shown were observed at the optimal or LD10 dose of the drugs when used alone or in combination. Table 7 shows the moderate activity of DTIC alone and adriamycin alone against L1210. The every 3 hours on Day 1 only schedule was used for adriamycin because of previous reports (12,13) that no alternative schedule was more effective for adriamycin treatment of this tumor. DTIC was given daily from Day 3 after tumor implantation through Day 17 or until death. Under these conditions of sequential treatment (adriamycin followed by DTIC) the combination was more than twice as effective in prolonging the lifetime of the mice than the most effective individual drug treatment.

Table 8 summarizes an experiment showing the superiority of DTIC plus adriamycin over single-drug therapy against B16 melanoma. Daily treatment was given from Days 2 to 9 after sc tumor implantation. The moderate advantage of concomitant combination chemotherapy was apparent with regard to the time required for the local tumor to reach a specified size as well as the median survival time of the mice. Table 9 illustrates the marked advantage of DTIC plus adriamycin against an early transplant generation of C3H mammary carcinoma. The combination was considerably more effective than individual drug treatment with regard to delay in primary tumor progression, median ILS, and number of tumor-free survivors.

In summary, DTIC has exhibited moderate activity against a number of mouse leukemias including sublines developed for resistance to methotrexate, 6-mercaptopurine, 6-thioguanine, and 5-FU. Activi-

TABLE 7.—DTIC + adriamycin: combination chemotherapy of ip implanted leukemia L1210 (10^5 cells)*

Mg/kg/dose				
DTIC (qd, Days 3-17)		Adriamycin (q3h, Day 1)		
Dose range	Optimal dose	Dose	Optimal dose	ILS (%)
108-500	300	—	—	55
—	—	0.65-3.0 (range)	1.08	61
65-300	180	1.08		122
65-300	180	0.65		138

*Data of Kline et al, Microbiological Associates, Inc. DTIC and adriamycin given ip.

TABLE 8.—DTIC + adriamycin: combination chemotherapy of sc implanted B16 melanoma*

Mg/kg/day				
DTIC†	Adriamycin†	T-C ‡ (days)	ILS (%)	Tumor-free survivors/ total No. treated
100	—	1	21	0/10
67	—	0	26	0/10
—	2.2	10	23	1/10
—	1.5	10	32	1/10
—	1.0	4	6	0/10
100	1.5	12	83	0/10
67	2.2	16	138	1/10
67	1.5	12	87	0/10

*Data of Griswold et al, Southern Research Institute.
†DTIC (ip) and/or adriamycin (sc) given qd, Days 2-9.
‡Time difference between treated (T) and control (C) tumors to reach a predetermined weight, usually 0.5 g.

TABLE 9.—DTIC + adriamycin: combination chemotherapy of sc implanted C3H mammary carcinoma*

Mg/kg/day				
DTIC†	Adriamycin†	T-C‡ (days)	ILS (%)	Tumor-free survivors/ total No. treated
100	—	17	54	0/9
67	—	14	73	0/10
—	2.2	17	59	1/10
—	1.5	10	0	0/10
—	1.0	8	0	0/10
100	1.0	42	>143	2/10
67	1.5	36	>170	4/10
67	1.0	28	138	1/10

*Data of Griswold et al, Southern Research Institute.
†DTIC (ip) and/or adriamycin (sc) given qd, Days 2-9.
‡Time difference between treated (T) and control (C) tumors to reach a predetermined weight, usually 0.5 g.

ty against solid tumors in rodents was variable depending on the tumor type. Of significance was the observation that DTIC provided a substantial increase in the survival of C3H mice with early generation transplants of C3H mammary carcinoma. DTIC, when combined with other active antitumor drugs, eg, cytosine arabinoside, cyclophosphamide, 5-FU, or adriamycin, provided a therapy which was superior to the individual drugs in the treatment of mouse leukemia L1210. The combination of DTIC plus adriamycin was superior to either drug used alone in the treatment of sc implanted B16 melanoma and C3H mammary carcinoma.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-

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Studies on the Mechanism of Action of DTIC (NSC-45388)[1]

Vincent H. Bono, Jr[2,*]

This paper will review the mechanism of action of DTIC, referring to the work of a number of investigators who have reported the salient features of its action.

DTIC itself is probably inactive. It is activated by light to diazoimidazole carboxamide (diazo-IC) which is a selective inhibitor of DNA synthesis in bacteria. However, this is probably not a physiologic activation. It is also activated by microsomal hydroxylation to yield carbonium ions which are capable of alkylating nucleic acids. However, it has not been shown that alkylation of nucleic acids is responsible for DTIC's biologic activity.

Both in cell culture (in the dark) and in vivo, DTIC inhibits RNA and protein synthesis more than DNA synthesis. It kills cells slowly and there appears to be no cell cycle phase of increased sensitivity. It slows the progression of cells through G_2 phase in vivo, and in vitro its most potent effect appears to be slowing the progression of cells through G_1 phase.

It is reasonable to propose that DTIC acts by the alkylation of as yet unknown target sites in the cell with carbonium ions, and that these target sites are probably more available in cells in early G_2 phase as well as in G_1 phase.

ACTIVATION OF DTIC

In 1970 Saunders and Schultz observed that DTIC was not cytotoxic to *Bacillus subtilis* when grown in the dark but was toxic when grown in the light (1,2). As discussed further by Loo et al (3), Saunders and Schultz found that light converted DTIC to diazo-IC which is highly cytotoxic (fig 1). In addition, diazo-IC spontaneously cyclizes to azahypoxanthine which, though toxic, is not as toxic as the diazo-IC itself. This suggests that diazo-IC has a more significant role than serving as a precursor to azahypoxanthine.

Yamamoto made similar observations in *Escherichia coli* (4). He observed that diazo-IC was approximately 1000 times more potent as an inhibitor of the growth of *E. coli* than its product azahypoxanthine. In addition, while hypoxanthine reversed the effect of azahypoxanthine, it did not reverse the action of diazo-IC. He found that at low concentrations (0.01 μg/ml) diazo-IC is selectively inhibitory to DNA synthesis.

In mammalian cell culture, DTIC was also much more potent when activated by light. Nevertheless it is active, at high concentrations, in the dark. A potential pathway for the metabolic activation of DTIC was described by Preussmann et al (5). They described the microsomal metabolism of phenyldimethyltriazene (fig 2). The pathway includes hydroxylation of one of the *N*-methyl groups producing an intermediate which decomposes in a stepwise fashion to produce formaldehyde, aniline, and finally a carbonium ion. Skibba et al (6) showed that this same pathway is taken by DTIC, the only difference being that aminoimidazole carboxamide (AIC) is produced instead of aniline. They showed that incubation of DTIC with rat liver microsomes produced approximately equal quantities of AIC and formaldehyde.

Mizuno and Humphrey (7) carried these studies further using ^{14}C-labeled DTIC. They used both

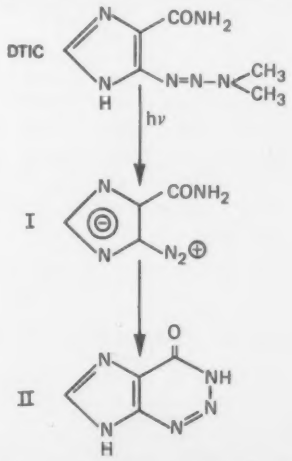

FIGURE 1.—Light-induced conversion of DTIC to 4-diazoimidazole-5-carboxamide (I) and 2-azahypoxanthine (II). Another product, dimethylamine, is not shown.

[1]DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide.

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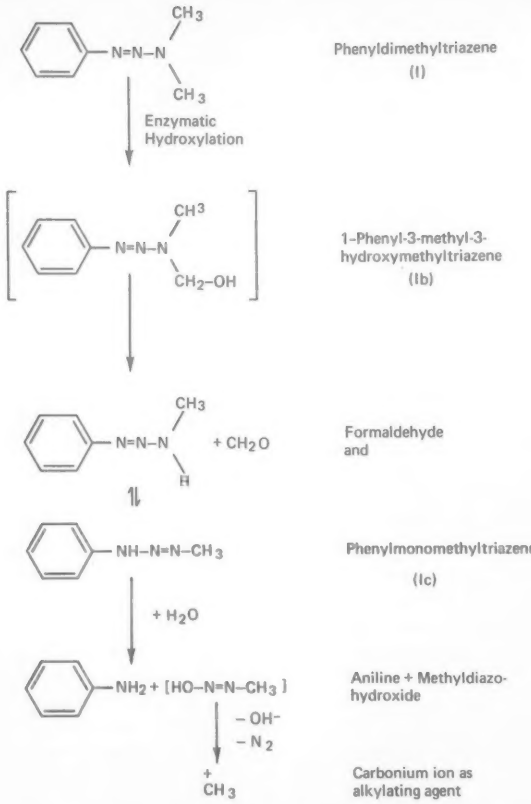

FIGURE 2.—Proposed activation mechanism of carcinogenic phenyldimethyltriazene (I) to form phenylmonomethyltriazene as proximate carcinogen and carbonium ion as ultimate alkylating agent. Formaldehyde and aniline as metabolites of (I) have been detected in the present study.

DTIC labeled in the *N*-methyl group and DTIC labeled in the 2-position of the imidazole ring. They showed that the methyl label appears in formaldehyde and that the ring label appears in AIC (table 1). When either RNA or DNA is included in the incubation it is methylated by methyl-^{14}C-DTIC, indicating the metabolic production of carbonium ions (table 2).

TABLE 1.—Metabolism of ^{14}C-labeled DTIC by Sarcoma 180 microsomal fraction

	Total cpm	
Labeled DTIC	Formaldehyde	AIC
Methyl-^{14}C	1256	0
2-^{14}C	0	1126

TABLE 2.—Alkylation of nucleic acids by DTIC*

	Cpm/mg of nucleic acid	
Tissue fraction	Methyl-^{14}C	2-^{14}C
Transfer-RNA	120	0
DNA	340	2
Ribosomal RNA	520	0

*Microsomes from Sarcoma 180 tumors were incubated with radioactive DTIC and calf thymus DNA for 4 hrs in the absence of light.

Alkylation of nucleic acids by DTIC was also shown to occur in mammalian cells in culture in the dark. Gerulath and Loo (8) found that the methyl groups of methyl-labeled DTIC alkylated the nucleic acid of Chinese hamster ovary (CHO) cells in culture in the dark.

SITES OF ACTION OF DTIC

Precise information on the sites of action of DTIC is not readily available. There are three kinds of studies that shed light on this question: (*a*) effect on macromolecular synthesis, (*b*) kinetics of its cytocidal action, and (*c*) effect on the progression of cells through the proliferative cell cycle. These will be discussed below and in each case the effects of DTIC that occur in the dark will be emphasized.

Effects on Macromolecular Synthesis

Shirakawa and Frei (9) studied the effects of DTIC on macromolecular synthesis in L1210 cells in culture over a 2-hour period at concentrations of up to 100 μg/ml. They found (in both light and dark) that RNA and protein synthesis are more sensitive to inhibition than was DNA synthesis (fig 3). These effects in the light are in contrast to the selective inhibitions of DNA synthesis seen by Yamamoto (4) in *E. coli* and by Saunders et al (2) in *B. subtilis*.

In recent studies by Wheeler (Southern Research Institute, personal communication), the light-activated DTIC was slightly more inhibitory to H.Ep. No. 2 cell DNA synthesis than RNA and protein synthesis after 26 hours' exposure to concentrations which reduce survival to 21% (fig 4). In the dark, this concentration, which was not cytocidal, inhibited protein synthesis. Curiously, it stimulated the incorporation of labeled thymidine but not uridine into DNA. No explanation is yet apparent.

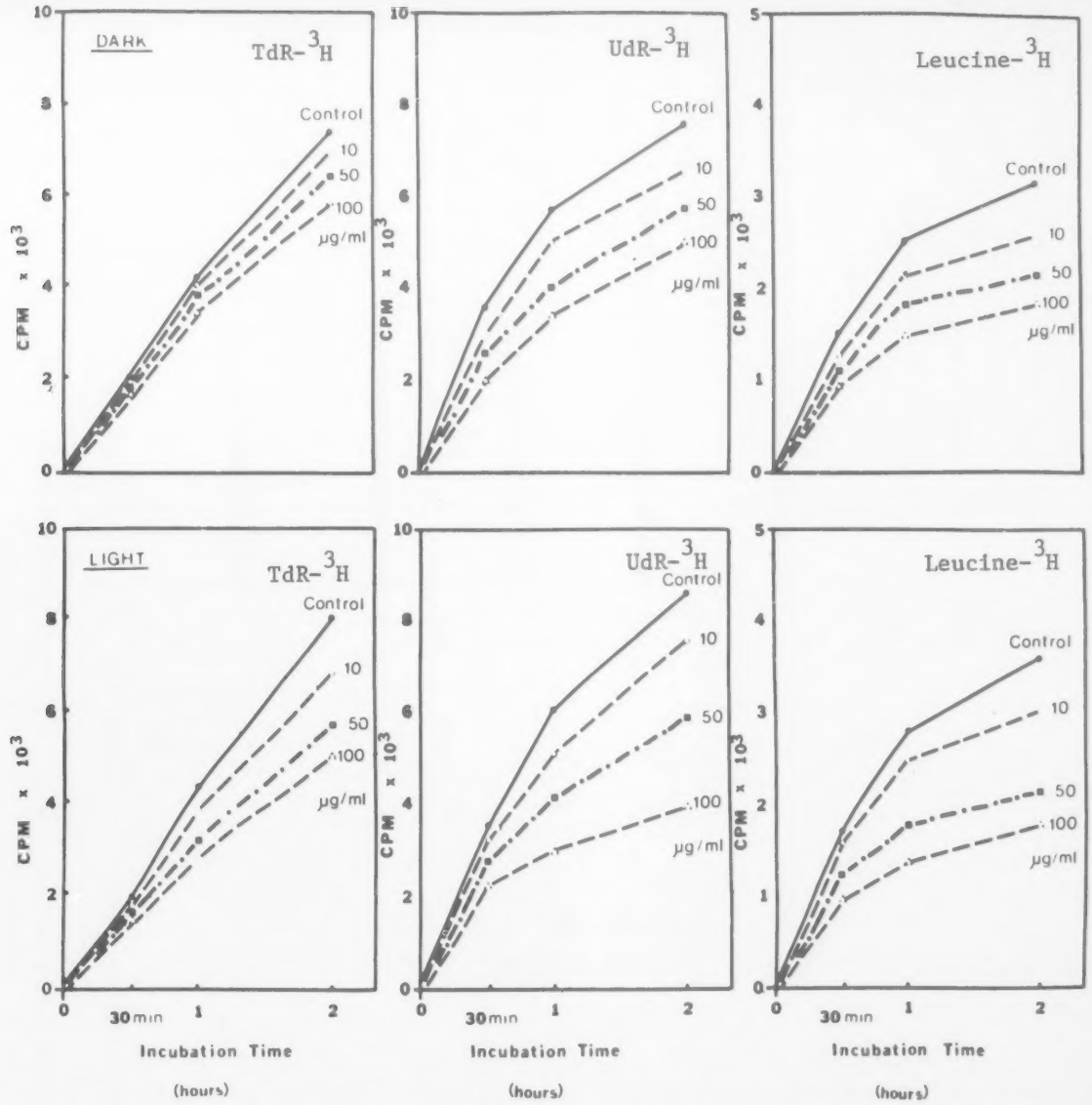

FIGURE 3.—Effect of DTIC upon incorporation of thymidine-^{3}H, uridine-^{3}H, and leucine-^{3}H into acid-precipitable materials of L1210 cells (sensitive in vitro). Each aliquot contained 2.21 × 10^6 cells/ml in 4 ml of Eagle's minimum essential medium with the indicated isotope and drug. Incubation was carried out at 37°C in water bath shaker (90 rpm) for the indicated period.

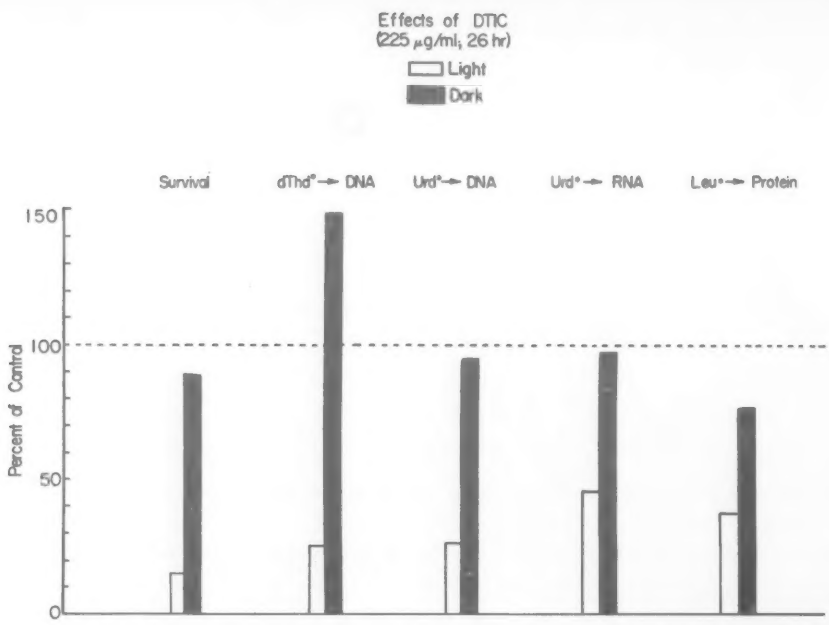

FIGURE 4.—Effect of DTIC (225 μg/ml for 26 hrs) on survival and macromolecular synthesis of H.Ep. No. 2 cells in vitro. Open bars = cells exposed to light during incubation period. Closed bars = cells kept in total darkness during incubation period.

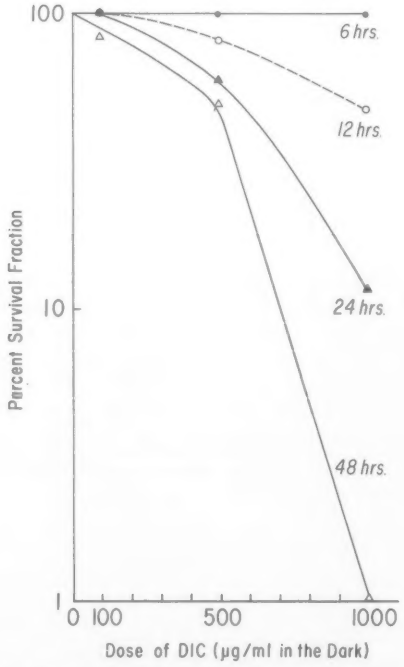

FIGURE 5.—Survival fractions of CHO cells following treatment with various concentrations of DTIC (in the dark) for different lengths of time (6, 12, 24, or 48 hrs) [*from* ref 10].

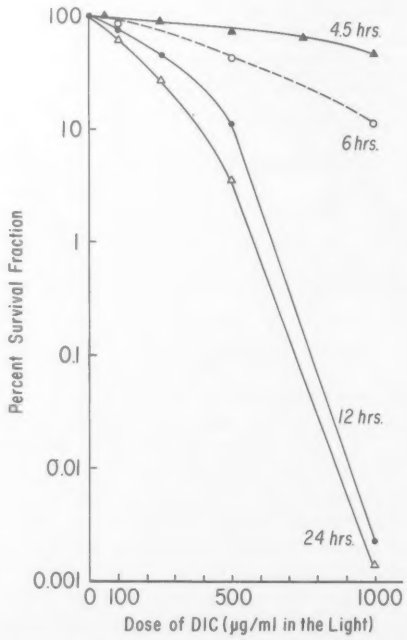

FIGURE 6.—Survival fractions of CHO cells following treatment with various concentrations of DTIC (in the light) for different lengths of time (4, 5, 6, 12, and 24 hrs) [*from* ref 10].

The results of both laboratories suggest that in the dark DTIC does not inhibit DNA synthesis selectively.

Kinetics of Cytocidal Action

The kinetics of the cytocidal effects of DTIC are shown in figures 5 and 6 from Gerulath et al (10). In CHO cells, DTIC in the dark required both high concentrations (500-1000 μg/ml) as well as long exposure times (24-48 hours) to reduce survival by 1-2 logs. The light-activated DTIC was more rapid in its onset of action and was active at lower concentrations. Since DTIC can reduce survival to from 1% to 10%, an implication of this data is either that it effectively kills cells in all phases of the cell cycle or that if it does kill in one phase preferentially, it does not prevent progression of cells into this sensitive phase. In other words, it would not have a self-limiting action. Similar cell kill kinetics have been reported for L1210 cells in vivo (fig 7) by Wodinsky et al (11). Cell cycle phase sensitivity was measured directly in synchronized CHO cells by Gerulath et al (10). No phase of increased sensitivity was observed when CHO cells were treated with 100 μg/ml for a 1- or 2-hour period (figs 8 and 9).

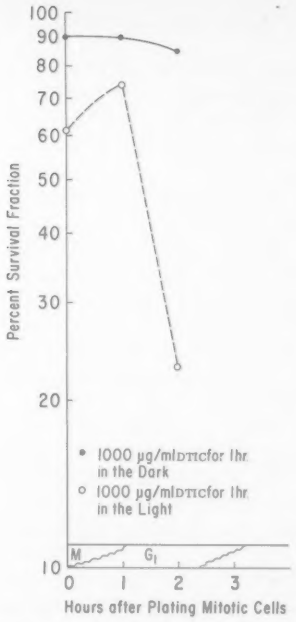

FIGURE 8.—Effect of DTIC on survival of synchronized CHO cells treated in M and G_1 phases of the cell cycle [*from* ref 10].

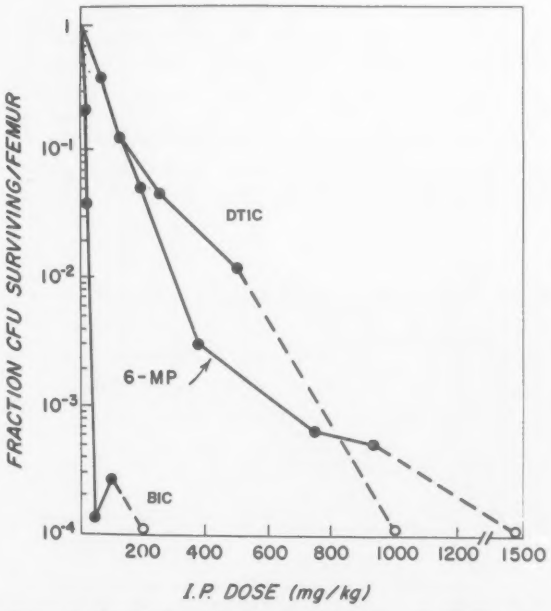

FIGURE 7.—Sensitivity of L1210 colony-forming cells (CFU) to 6-MP, DTIC, and BIC. Broken lines are extensions of the survival curves to next highest dose tested at which no spleen colonies were observed. Each point is the average of 2 or 3 replicate experiments; 10 mice per experiment.

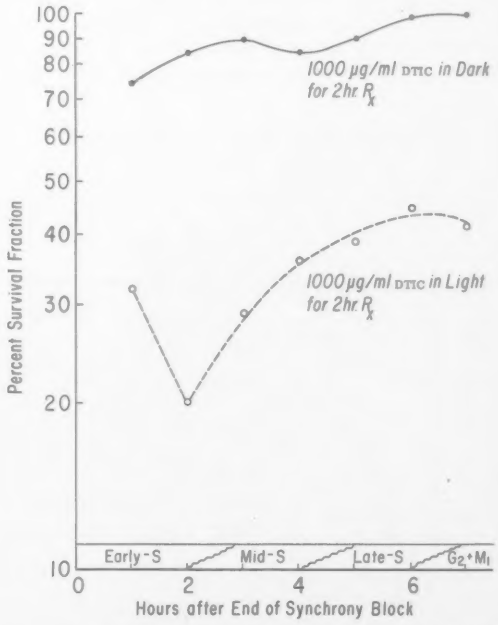

FIGURE 9.—Effect of DTIC on survival of synchronized CHO cells treated in S and G_2 phases of the cell cycle. Rx = treatment [*from* ref 10].

Cell Cycle Progression Effects

Studies on the effect of DTIC on CHO cell cycle progression by Gerulath et al (10) have shown that DTIC in the dark has no effect on the progression of G_2 cells into mitosis, when cells are within 2 hours of mitosis (fig 10). By contrast, light-activated DTIC has an immediate effect on the G_2-M phase transition. DTIC (in both light and dark) delays but does not stop the transition of S-phase cells into G_2 phase (fig 11). Of interest is that DTIC in both light and dark appeared to stimulate the transition of half the G_1 cells into S phase but stopped the transition of the other half (fig 12).

In studies on cell cycle progression in L1210 cells in vivo by Shirakawa and Frei (9) the most significant effect was a slowing of cell progression through G_2 (fig 13). In this percent labeled mitosis (PLM) curve the time to half maximum PLM measures the duration of G_2 phase and is increased from a control of 2 hours to about 4-5 hours. The duration of S phase, the time from half-height-up to half-height-down, is 9 hours in control and 11 hours in DTIC-treated mice. The progression effect on G_2 phase was dose dependent and at doses of 500 mg/ml virtually no cells progressed into mitosis.

In studies in my laboratory[3] using flow microfluorometry, the effect on cell cycle progression was evaluated in exponentially growing L1210 cells in vitro (fig 14). The DNA content distribution of control cells shows a peak of cells with a G_1 DNA content, a small peak of G_2 cells, and a plateau of cells in S phase with intermediate DNA contents. At 100-μg/ml continuous exposure for 24 hours in the dark, cells grew at a rate of 60% of control. The primary effect seen was an accumulation of G_1 phase cells indicating a prolongation of G_1 phase. At 233 μg/ml, a dose reducing cell growth to 27% of control over 24 hours in the dark, there was an accumulation of G_2 phase cells indicating a

[3]Dion RL, and Bono VH, Jr. Personal communication, NCI.

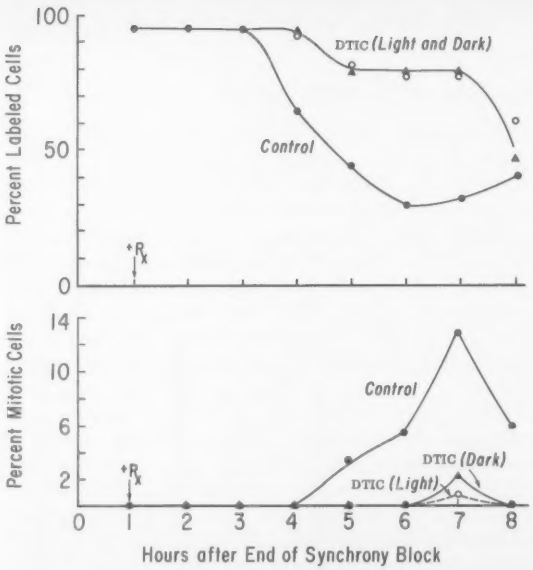

FIGURE 11.—Effect on cell progression from S to G_2 and M phases during continuous treatment with DTIC (1000 μg/ml) [*from* ref 10].

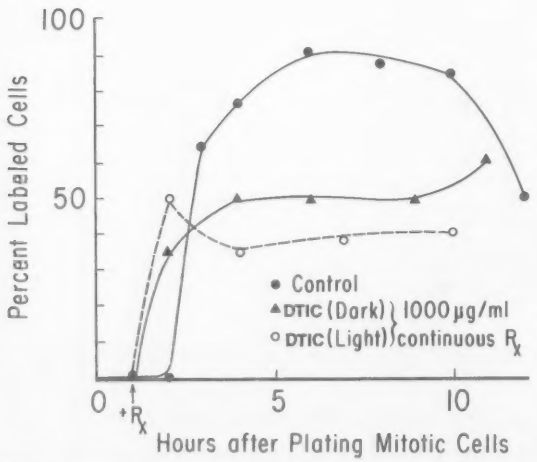

FIGURE 10.—DTIC effect on cell progression from G_1 into S phase [*from* ref 10].

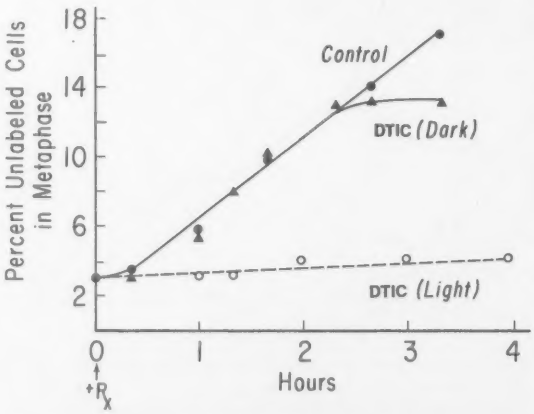

FIGURE 12.—Effect on cell progression from G_2 into M phase during continuous treatment with DTIC (1000 μg/ml) [*from* ref 10].

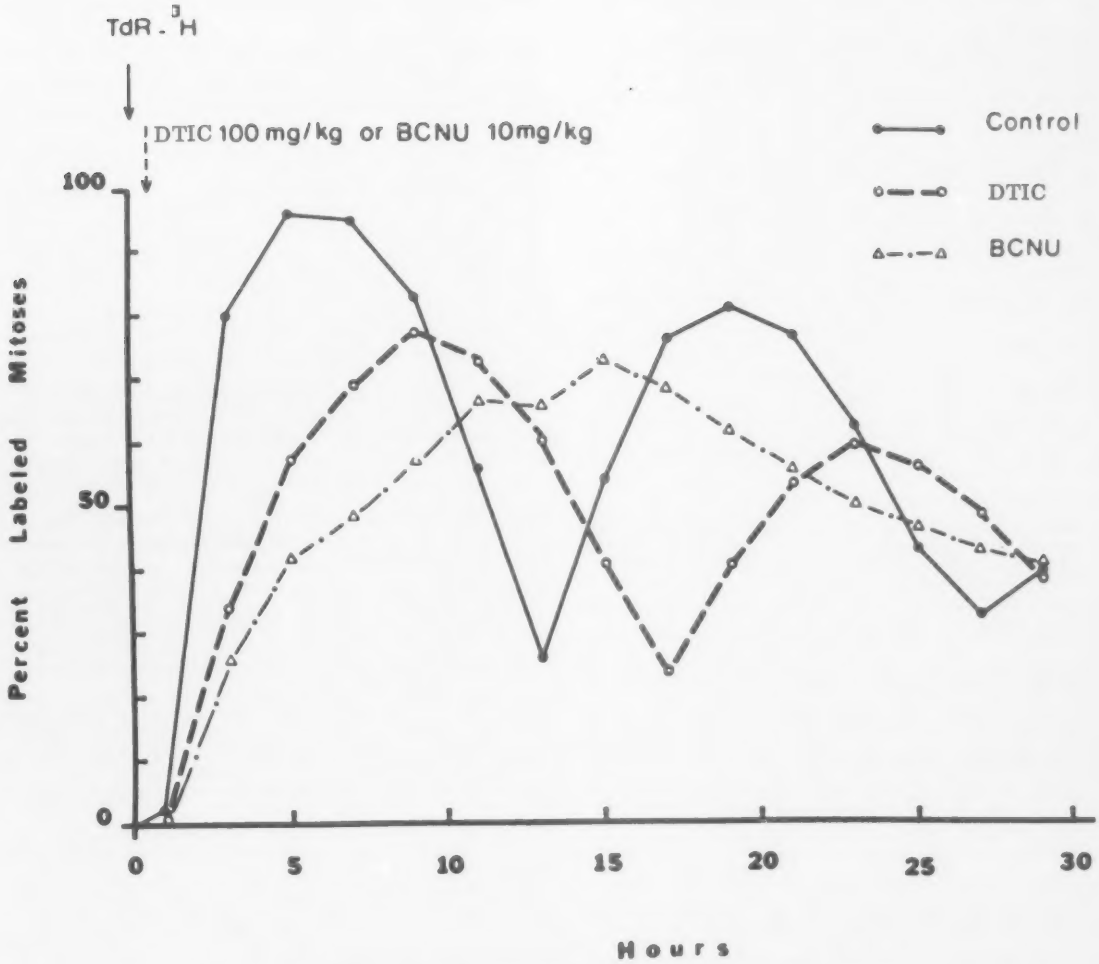

FIGURE 13.—Effect of DTIC and BCNU on labeled mitosis curve.

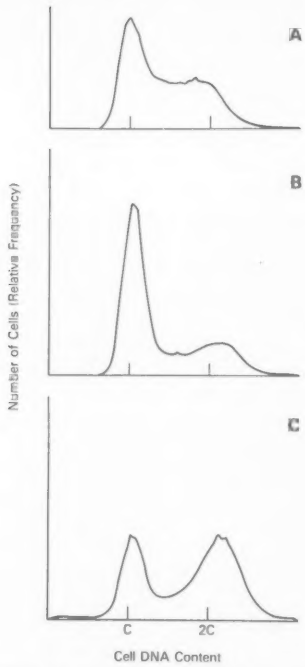

FIGURE 14.—Effect of DTIC on the cell DNA content frequency distribution of L1210 cells in vitro: A = control; B = 100 μg/ml for 24 hrs (growth rate 60% of control); C = 233 μg/ml for 24 hrs (growth rate 27% of control).

prolongation of G_2 phase. At low concentrations the primary effect was on G_1 cell progression and at higher concentrations an additional effect on G_2 cell progression was seen. There appears to be no effect on the ability of cells to pass through S phase at either concentration.

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Mechanism of Action and Pharmacology Studies With DTIC (NSC-45388)[1,2,3]

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DTIC is an antitumor agent whose mechanism of action has been elucidated in light of its pharmacology. We have previously reported on the disposition and metabolism of DTIC (1-4). After iv administration of the drug to patients, its disappearance from the plasma is biphasic, with an initial half-life of 19 minutes and a terminal half-life of 5 hours (fig 1). In a patient with renal and hepatic dysfunctions, the half-lives are lengthened to 55 minutes and 7.2 hours (fig 1, dotted line). The average cumulative excretion of unchanged DTIC in the urine is 40% of the injected dose in 6 hours. In comparison, in the dog, the initial plasma half-life of DTIC is 6 minutes and the terminal half-life is 1.5 hours (fig 2); however, the 6-hour cumulative urinary excretion is < 20%. These observations suggest that DTIC is more quickly degraded in the dog than in man. In the rat, DTIC is concentratively excreted in the bile to attain a concentration twice as high as that in the plasma at steady state, although < 3% of the injected dose is so excreted in 2 hours. At therapeutic concentrations DTIC is not appreciably bound to human plasma protein. In the dog it enters the central nervous system (CNS) only to a limited extent (1); presumably its entry into human CNS is likewise restricted.

In man, dogs, and mice, DTIC is extensively degraded. In these species, besides unchanged DTIC, 5-aminoimidazole-4-carboxamide (AIC) is a major metabolite of DTIC excreted in the urine (3,4). AIC is not derived endogenously but from the injected DTIC because the administration of radioactive DTIC labeled with ^{14}C in the imidazole portion of the molecule (DTIC-2-^{14}C) gives rise to AIC-2-^{14}C. Thus the in vivo degradation of DTIC to AIC reverses the mode of formation of DTIC (5). In mice injected ip with radioactive DTIC uniformly labeled with ^{14}C in the *N*-methyl groups (DTIC-DM-^{14}C), 10% of the dose is recovered as $^{14}CO_2$ in the expired air. However, with DTIC-2-^{14}C, the expired CO_2 is not radioactive (4). Clearly, one or both of the *N*-methyl groups have undergone oxidation to CO_2 but the imidazole ring remains intact.

The cytotoxicity of DTIC to *Bacillus subtilis* is greatly enhanced when the culture is exposed to light during incubation (6). This enhancement in lethality has also been seen in cultured human melanoma cells and Chinese hamster ovary (CHO) cells (7). Any proposed mechanism of action of DTIC must be able to accommodate this observation, as well as the above-mentioned facts that AIC is an in vivo metabolite of DTIC and that one or both of the *N*-methyl groups of DTIC are oxidized to CO_2.

To elucidate the molecular mechanism of cytotoxic action of DTIC, melanoma or CHO cells have been incubated with radioactive DTIC in the presence and absence of light (7). After incubation, the cells are harvested and lysed, and the cellular nucleic acids are isolated and subjected to CsCl gradient centrifugation. With DTIC-DM-^{14}C in the dark, there appears a slight but unmistakable peak of radioactivity corresponding to the density of DNA. To confirm that this peak is really located in the DNA fraction, double-labeling experiments have been performed: CHO cells are treated with tritiated thymidine before exposure to DTIC-DM-^{14}C. The peak of ^{14}C radioactivity derived from DTIC-DM-^{14}C coincides exactly with that of tritiated thymidine incorporated in the DNA. However, when these experiments are repeated in the light, this peak of ^{14}C radioactivity is absent.

With DTIC-2-^{14}C and CHO cells, the same results are obtained either in the presence or the absence of light. The highest radioactivity is always associated with the highest density corresponding to the position of RNA on the gradient. In addition, a

[1]Supported by contract N01-CM-61156 (Division of Cancer Treatment) and in part by Public Health Service grants CA-05831 and CA-11520 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

[3]Dedicated to the late Jeffrey A. Gottlieb, MD, who pioneered the combination chemotherapy of sarcomas with DTIC and adriamycin with notable success.

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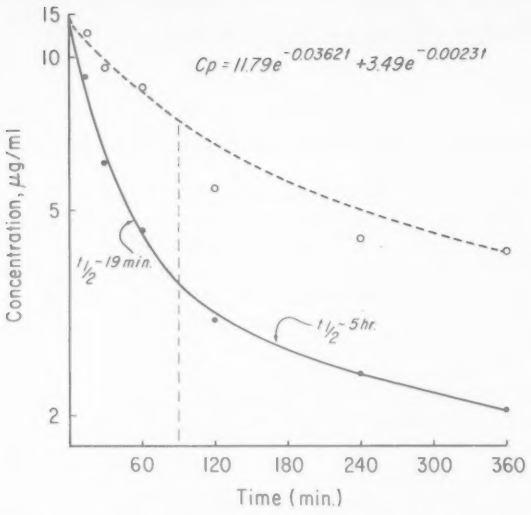


FIGURE 1.—Plasma disappearance of DTIC in 3 patients; dose = 250 mg/m² iv.

small but unequivocal peak of radioactivity is also detected in the DNA fraction, which becomes more pronounced when a higher concentration of DTIC-2-^{14}C is employed. Again, pretreatment with tritiated thymidine confirms that this small peak is located at the DNA density.

To delineate further the fate of DTIC-2-^{14}C in CHO cells, we have analyzed chromatographically the contents of disrupted cells on Sephadex G-50 columns. The percentage of the isotope associated with cellular macromolecules is higher in cells incubated with DTIC-2-^{14}C in the dark than in light. But regardless of the presence or absence of light, small amounts of AIC and 2-azahypoxanthine (aza-Hx) have been detected in cells (8).

These findings can be interpreted in light of the current understanding of the biotransformation of 1-phenyl-3,3-dimethyltriazene (9). DTIC readily undergoes photodecomposition to 5-diazoimidazole-4-carboxamide (DZC) and dimethylamine. A reactive electrophile, DZC shows a strong affinity for nucleophilic groups. In fact, aza-Hx evidently arises from intramolecular cyclization involving the 4-carboxamide moiety and the 5-diazo group of DZC; however, the role of aza-Hx is unclear, for evidence is lacking that aza-Hx possesses any biologic activity (10). Dimethylamine, the other product of the photodecomposition of DTIC is not cytotoxic at the level attainable after a therapeutic dose of DTIC. The alternative biotransformation pathway of DTIC consists in the loss of an *N*-methyl group, ultimately as CO_2, by oxidative *N*-demethylation. The 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MIC) thus formed rearranges to 5-(3-methyl-2-triazeno)imidazole-4-carboxamide, which subsequently decomposes to AIC and the precursor of the methyl carbonium ion. The biochemically and pharmacologically active entity of this mode of biotransformation of DTIC is likely the methyl carbonium ion or its precursor.

There is little doubt that the photodecomposition pathway is operative at all times. Even in vivo when light is absent, the generation of DZC from DTIC never entirely ceases but is merely reduced in rate sufficiently to permit the reactions of the alternative pathway to take place.

The direct contributions of DZC to the cytotoxicity of DTIC are uncertain. On one hand, though reactive, DZC may experience difficulty in entering cells as a dipolar molecule. On the other hand, when produced rapidly in quantity by photodecomposition, some DZC must find its way inside the cells to interact with cellular macromolecules; the exact nature of this interaction has not been elucidated. It is unlikely that DZC can give rise to AIC through enzymatic reductive cleavage. First, chemically a diazo compound differs fundamentally from an azo compound, and it is inconceivable that mammalian azo reductase (EC 1.6.6.7) is so lacking in specificity as to fail to distinguish a diazo from an azo linkage. Second, even allowing for the promiscuity of azo reductase to reduce DZC to AIC, the existence of this enzyme in CHO cells remains to be demonstrated. In comparison, however, *N*-demethylase activity is detectable in these cells as evidenced by the liberation of radioactive

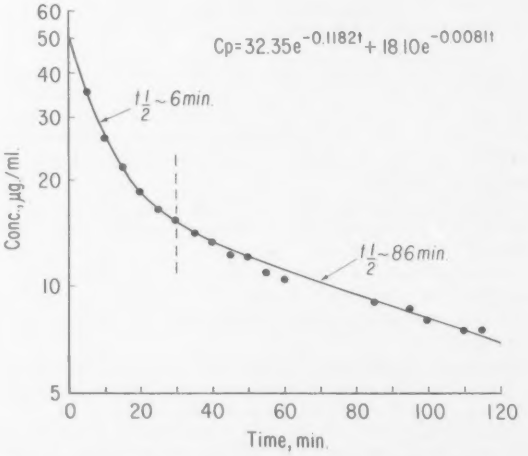
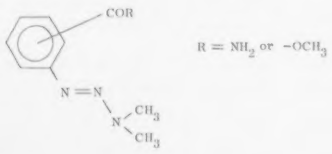


FIGURE 2.—Plasma disappearance of DTIC in 6 dogs; dose = 20 mg/kg iv.

CO_2 upon incubation of CHO cells with DTIC-DM-^{14}C in the dark; as predicted, DTIC-2-^{14}C does not give rise to $^{14}CO_2$ under these conditions (7).

Overall, the presence or absence of light is really immaterial, for without fail, enough DZC will be produced from DTIC to interact with cellular macromolecules. This is consistent with our observation that the radioactivity of DTIC-2-^{14}C is invariably associated with both DNA and RNA, although more so in light. However, in the dark, the photodecomposition of DTIC to DZC is hindered but not entirely stopped, such that the alternative pathway also becomes allowed, eventually leading to the generation of the methyl carbonium ion. Apparently, in cultured CHO cells DZC interacts with DNA and RNA, but the methyl carbonium ion limits its attack to DNA only.

To investigate the structure-activity relationships among the triazenes, we have synthesized a series of phenyltriazenes of the general formula (11):



At their optimal doses, these phenyltriazenes are as effective as DTIC against ip implanted mouse L1210 leukemia. From this finding, and the finding that triazeno derivatives of *v*-triazole and pyrazole are equally active, we may conclude that the imidazole ring of DTIC may be replaced with other aromatic systems (12,13) without causing a loss of antitumor activity. Further, antitumor activity is retained if a $-CO_2CH_3$ group is substituted for the $-CONH_2$ group. Finally, it is not essential that the annular $-CONH_2$ or $-CO_2CH_3$ be adjacent to the triazeno side chain, since in the phenyltriazenes, the o–, p–, and m– analogs display comparable antitumor activity.

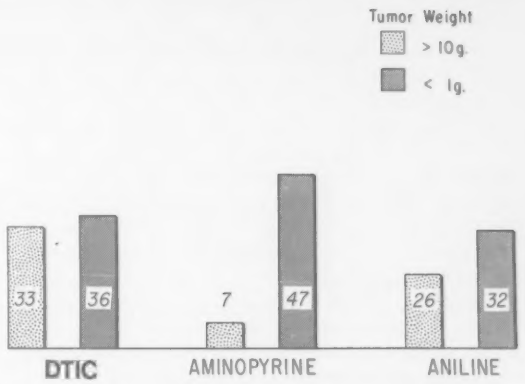


FIGURE 3.—Drug metabolism by liver microsomes of Sprague-Dawley rats bearing Walker 256 carcinosarcoma: % of control.

Clinicians treating patients with cancer must realize that neoplasia may influence drug disposition and metabolism markedly. This is certainly true in experimental animals (14,15). In support of these findings, we have observed that after DTIC-DM-^{14}C administration, mice implanted with ip L1210 leukemia produce less $^{14}CO_2$ than do healthy animals (4). Similarly, the hepatic microsomal enzyme hydroxylation of aniline, *N*-demethylation of aminopyrine, and *N*-demethylation of DTIC are

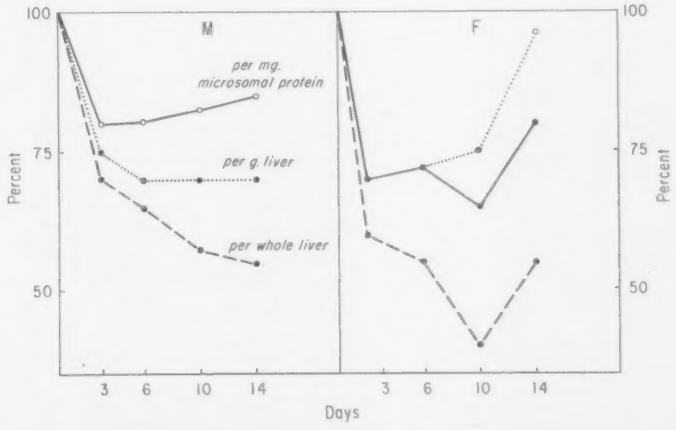


FIGURE 4.—Rat hepatic microsomal DTIC-demethylase activity after injection of the Pasteur strain of BCG; dose = $6 \times 10^8/m^2$ iv.

impaired in Sprague-Dawley rats bearing the Walker 256 carcinosarcoma, especially when the tumor is large (J. Yoshimura and T.L. Loo, unpublished data) (fig 3). It is entirely conceivable that the metabolism of DTIC is also impaired in patients with cancer. If the only requisite for DTIC to be active is the production of the methyl carbonium ion, then the inhibition of the *N*-demethylation of DTIC, the first step toward CH_3^+ generation, will result in apparent underdosing.

Anticancer agents are seldom used alone but rather in combination with other anticancer agents, as supportive therapeutic agents, and as adjuvants. Because of this, drug interactions among these agents and adjuvants deserve serious consideration. Recently we have reported (16) that the iv injection of the Pasteur strain of bacillus Calmette-Guérin (BCG) causes a significant reduction in hepatic microsomal aniline hydroxylase and DTIC *N*-demethylase activities in Sprague-Dawley rats (fig 4; only those values represented by solid circles are statistically significantly lower than those of the control). These findings have important clinical implications, particularly since DTIC and BCG are currently used in combination for the chemoimmunotherapy of a number of neoplastic diseases.

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Natural History of Malignant Melanoma as Related to Therapy

Vincent T. DeVita, Jr[1,*] and Richard I. Fisher[2]

The natural history of malignant melanoma is complex, making interpretation of therapeutic experiments difficult. This is in no small way due to the fact that the cell of origin, the melanocyte, may reside almost anywhere in the body and the pattern of dissemination and response to treatment and the probability of survival vary markedly with the primary site.

Melanomas make up 1%-3% of all tumors, which makes them uncommon but not rare (1). They occur more often in males than females (1.3 to 1) and are more commonly seen in countries, or areas of countries, where exposure to sunlight is more intense (2). The most common primary sites are the head and neck and the trunk regions in males, and the distal lower extremities in females, a fact which also suggests a causal role for solar exposure to exposed areas (3,4). This observation is enhanced by noting that the head and neck region and the genitalia are the two areas with the greatest density of melanocytes (5) (a factor which might be expected to influence the incidence), yet the incidence of melanoma is far greater in the head and neck region which, until recently, was far more likely to receive greater exposure to sunlight.

Pathology

Malignant melanoma occurs in three distinct clinicopathologic forms as illustrated in table 1 (6). Lentigo maligna melanoma is a slowly evolving, tan, freckle-like lesion, 1-2 cm in diameter occurring on exposed cutaneous surfaces in elderly people. The premalignant phase, lentigo maligna, may exist for many years before invasion develops. The bizarre pleomorphic malignant melanocytes scattered among normal cells in the basal layer characterize the lesion.

Superficial spreading melanoma is usually a large lesion 2-3 cm in diameter occurring anywhere on the body in middle-age persons. The margins of the lesion are often elevated and combinations of colors such as tan, brown, black, or rose characterize the lesion. Although it may evolve slowly, the time course of the disease is less indolent than lentigo maligna melanoma.

Nodular melanoma is the most aggressive lesion, progressing over months to years in some cases and occurring as spherical berry-like nodules on most body surfaces. Pathologically, no isolated intraepidermal phase occurs without dermal invasion.

Superficial spreading and nodular melanomas are by far the most common types of tumor seen by physicians (fig 1).

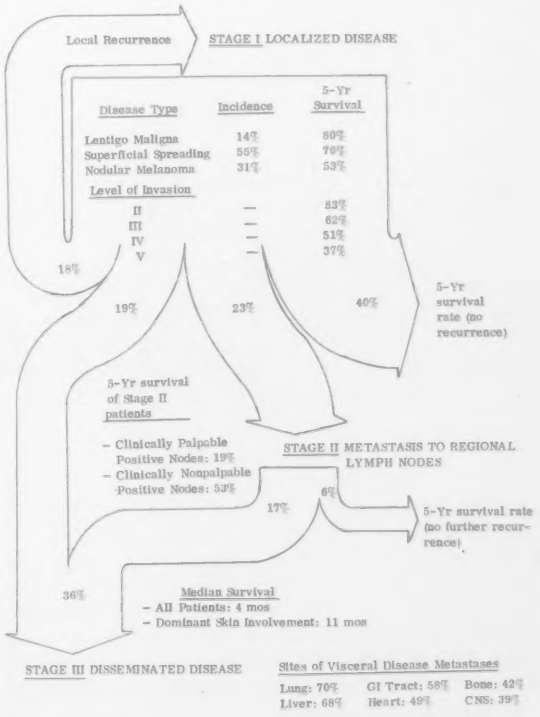

FIGURE 1.—Natural history of cutaneous malignant melanoma.

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TABLE 1.—Clinicopathologic forms of malignant melanoma

Type of melanoma	Location	Median age	Sex predilection	Duration	Histologic characteristics of non-invasive areas
Lentigo maligna	Exposed surfaces	70	None	5-15 yrs	Pleomorphic normal and bizarre melanocytes in basal regions
Superficial spreading	All surfaces	56	Lower legs in women	1-5 yrs	Intraepidermal monomorphous malignant melanocytes
Nodular	All surfaces	49	None	Months-2 yrs	Intraepidermal and dermal invasion occur simultaneously

General Factors Influencing Prognosis

Although the level of invasion and stage of tumor are clearly the most important information influencing selection of treatment and ultimate prognosis (see below), they should not be used to design or interpret clinical trials in the absence of the other factors which can influence the outcome of a study.

The most important of these influences are sex and primary site in patients with resectable tumors, and sex and dominant site of involvement in patients with metastases (3,7,8). The prognosis in women, stage by stage, treatment by treatment, is consistently better than in men. Thus, some form of endocrine control over this tumor has been postulated and hormonal manipulation attempted (2).

Distal extremity lesions have a better prognosis than those of the trunk and head and neck (9). The data suggest that the time to appearance of metastases in distal extremity lesions is longer than that for the head and neck region (2). In the former, metastases appear in an average of 1 year while in the latter the median time for the appearance of metastases is 2 years. Therefore, not only do women have a superior prognosis when considering identical primary sites, but they are more likely to have distal extremity lesions, which have a better prognosis regardless of sex. The influence on the interpretation of data of an unbalanced randomization by sex alone is readily apparent.

These points are most important in adjuvant studies in patients with localized disease. Early interpretation of data may lead to the false impression of a favorable influence on the time to tumor recurrence when the outcome is more related to the sex distribution and site of involvement of the population in question.

In patients with metastatic tumor, visceral dominant disease carries a worse prognosis than skin dominant lesions (fig 1). In the latter instance visceral metastases eventually develop and are the cause of death. Again, sex influences drug response. Women and patients with soft tissue disease are more likely to respond to drug therapy (and very likely immunotherapy) than those with most visceral metastases (8).

For many years chemotherapists have been plagued by the observation that the first one or two patients with metastatic melanoma treated with a new drug often responded. Thereafter, few additional responses were noted in the remainder of the study. This observation is probably related to the superior prognosis, and greater likelihood of response, of patients with skin dominant lesions who are more often available in clinics when new drug or immunotherapy trials are initiated.

A few other points about sites of metastases are worthy of note. Melanoma is one of the few tumors which consistently metastasizes to the spleen and may occasionally cause unexplained splenomegaly (2). The heart, another uncommon site of metastases for most tumors originating outside the chest, is also frequently involved by malignant melanoma cells and significant clinical arrhythmias may occur and influence the outcome of the patient's course. Malignant melanoma is one of the most common metastatic malignant tumors leading to unexplained small bowel obstruction from metastases to the small bowel mucosa. Intestinal obstruction also frequently complicates the course of patients undergoing systemic treatment for metastatic disease.

Other sites of metastases are illustrated in figure 1. By far the most important is involvement of the central nervous system. Autopsy series demonstrate central nervous system involvement in 36% (2) to 54% (8) of patients. Although involvement of the brain is occasionally localized and will occasionally respond to surgical resection alone, most patients have multiple solid metastases. Even minor neurologic symptoms in patients known to

have malignant melanoma should be considered significant since they often presage development of overt central nervous system involvement. In general, as with most tumors, those patients with central nervous system metastases do poorly with systemic treatment alone.

The phenomenon of spontaneous regression of melanoma has been the subject of several reports. In a compilation of cases of spontaneous regression of cancer by Everson and Cole (11), melanoma was one of the most common tumors reported. Considering the low incidence of the disease, these data suggest some unique characteristic of malignant melanoma, perhaps immunologic, which makes patients with metastatic tumor more susceptible to spontaneous regression. Although the frequency of spontaneous regression varies, it is not likely to be in excess of 1%. We have observed only one possible spontaneous regression of tumor in over 150 patients with metastatic malignant melanoma treated in the Medicine Branch, DCT, NCI.

Level of Invasion and Staging

In 1969 Clark et al studied the relationship of survival data to depth of invasion of primary malignant melanoma (12). They defined five levels of invasion as shown in table 2. McGovern has also confirmed that the depth of invasion is an important prognostic indicator in patients with melanomas (13). Table 3 correlates 5-year survival rates with the level of invasion and histologic type. Survival varies from 85% for level II to 23% for level V. The greater virulence of nodular melanoma compared to superficial spreading melanoma for a given level of invasion probably reflects the greater likelihood of nodal involvement.

Several staging classifications are in use for malignant melanoma, often adding further confusion to the interpretation of data. A simple, commonly used example of a staging classification is shown in table 4.

There is little convincing evidence that nodal resection by itself influences the prognosis of patients with or without nodal involvement. As in breast cancer, knowledge of the status of the draining lymph nodes gives the physician a powerful indication of the prognosis and influences his selection of treatment. Involvement of lymph nodes markedly reduces the likelihood of survival at 5 and 10 years (fig 1). Patients with palpable nodes involved by tumor have a worse prognosis (10%-19% 5-year survival) than patients with nodes involved only microscopically (30%-53% 5-year survival) (7,10). This fact should always be strongly considered in surgical adjuvant studies.

Prime candidates for adjuvant chemoimmunotherapy trials are patients with stage II disease, regardless of level of invasion, and patients with level IV or V involvement, regardless of nodal status. Until recently, little data were available correlating level of invasion with nodal involvement and the survival of patients with various levels of invasion with and without positive nodes. Recently Wanebo et al (14) have made these correlations. In patients with clinical stage I disease (nonpalpable lymph nodes) with lesions confined to the extremities, the incidence of nodal involvement at lymphadenectomy increased progressively with level of invasion and 5-year survival decreased commensurately (table 5). Table 6 demonstrates that the overriding influence on survival for patients with a given level of invasion of an extremity melanoma is the status of the regional lymph nodes determined at an elective lymphadenectomy. There is little difference in survival among patients with level II, III, or IV invasion in the ab-

TABLE 2.—Level of invasion in malignant melanoma

Level	Characteristics
I	All tumor cells above basement membrane
II	Invasion into loose connective tissue of papillary dermis
III	Tumor cells at junction of papillary and reticular dermis
IV	Invasion into reticular dermis
V	Invasion into subcutaneous fat

TABLE 3.—Correlation of level of invasion and histologic type in malignant melanoma [*from* McGovern (13)]

	% 5-yr survival for—	
Level	Superficial spreading	Nodular
II	81	85
III	65	39
IV	56	45
V	50	23

TABLE 4.—Clinicopathologic staging of malignant melanoma

Stage	
I	Localized primary melanoma
IA	Local recurrences
II	Metastases to regional lymph nodes
III	Disseminated melanoma

TABLE 5.—Clinical stage I patients with lesions on the extremities: relationship of level of invasion to incidence of nodal involvement [*from* Wanebo et al (14)]

Level of invasion	No. of patients	Patients with positive nodes (%)	5-yr survival (%)
II	28	4	100
III	62	7	88
IV	69	29	66
V	10	70	15
	169		

sence of identifiable tumor in the draining lymph nodes. Thus, the level of invasion is important and should be determined on all patients since it does predict for lymph node involvement. It also may identify patients in level V or patients with blood vessel invasion, both of whom may have a high risk of recurrence even in the absence of lymph node invasion. However, knowledge of the status of lymph nodes themselves appears to be the most important piece of information necessary for the design of the clinical trial. Further data of this type are necessary to corroborate the relative importance of level of invasion in predicting stage of disease.

Conclusion

Like most tumors, malignant melanomas are occasionally localized and cured by resection of the primary lesion alone. The actual impact of treatment modalities on survival, including the extent of resection of primary lesion, lymphadenectomy, local limb perfusion, adjuvant chemotherapy and/or immunotherapy, or chemotherapy for advanced metastatic disease, is clouded by a failure to appreciate or acknowledge the variables known to influence the prognosis of a patient with malignant melanoma. Unlike most tumors originating in a geographically confined single organ system, the initial biologic behavior of malignant melanoma is as variable as the location of the primary site.

The flow of a typical population of patients with clinical stage I malignant melanoma taken from grouped large series of patients in the literature is illustrated diagrammatically in figure 1. Also shown are the incidence and survival by histologic type and level of invasion.

Of all patients with stage I melanoma who undergo primary resection with or without lymphadenectomy, about 40% will survive 5 years free of recurrence. This figure will obviously vary from series to series depending on the mix of patients with the variables mentioned above. Twenty-three percent of clinical stage I patients may have lymph node invasion at the time of diagnosis of the primary (determined by lymphadenectomy) or may develop nodal metastases later. One quarter of these patients (6% of the total population in fig 1) will be alive and free of disease at 5 years while three quarters (17% of the total population in fig 1) will develop evidence of dissemination. Nineteen percent of patients with localized disease will develop subsequent evidence of systemic disease (stage III) without ever presenting as stage II disease. The remaining 18% of resected patients will develop evidence of local recurrence and can be

TABLE 6.—Disease-free survival in 133 patients with extremity melanomas, clinical stage I, correlated with level of invasion and nodal involvement [*from* Wanebo et al (14)]

Level	Total No. of patients	Patients with positive nodes	5-yr survival (%)	Patients with negative nodes	5-yr survival (%)
II	19	1	100	18	100
III	46	2	100	44	91
IV	44	11	27	33	82
V	4	3	0	1	0
	113	17		96	

recycled through the diagram. Essentially all stage III patients will die of their disease.

The data in figure 1 illustrate the points made earlier: the disease is curable if localized, the level of invasion predicts nodal metastases and survival, and nodal invasion is the most important influence on the likelihood of dissemination. However, it must be remembered that in many series complete data on all the prognostic variables were not available and often the influence of sex, age, and site of involvement was not taken into account.

In the future, investigators must recognize that a great amount of information on the natural history of malignant melanoma is available. Therapeutic trials can only be meaningful if patients with similar prognoses are studied in a prospective randomized manner.

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Surgical Treatment of Malignant Melanoma

Steven A. Rosenberg[1,*]

SUMMARY

Surgery alone is incapable of curing most patients with malignant melanoma. Wide local excision and skin grafting are the most important factors in the local treatment of malignant melanoma. Regional lymph node dissection in selected patients with clinical stage I disease is useful in identifying those patients with positive lymph nodes suitable for studies of adjunctive treatments. Studies of the role of bacillus Calmette-Guérin in the treatment of primary malignant melanoma and stage II malignant melanoma are currently in progress in the Surgery Branch of the National Cancer Institute.

[Cancer Treat Rep 60:159-163, 1976]

Despite the aggressive application of modern surgical techniques the results of the surgical treatment of malignant melanoma are poor. Of all patients with a diagnosis of malignant melanoma who consult a physician > 60% will ultimately die from the disease (1). In the majority of patients with apparently localized malignant melanoma at the time of diagnosis, microscopic dissemination beyond the local site has already occurred.

Adjunctive treatment modalities such as chemotherapy and immunotherapy in combination with surgery offer hope for improved treatment. A modern approach to the surgery of malignant melanoma must be conditioned by the fact that adjunctive therapies will be necessary in those patients identified to have a poor prognosis.

The role of surgery in the treatment of malignant melanoma will be considered separately for each clinical stage of the disease. The clinical staging criteria used at the National Institutes of Health are presented in table 1. Clinical stage I melanoma is confined solely to the local site. A separate category, IA, for local recurrence is included. In clinical stage II, clinical evidence of regional lymph node metastases is present. In clinical stage III disease, clinical evidence of dissemination to visceral organs or distant lymph nodes, or distant cutaneous metastases are evident.

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STAGE I

The treatment of clinical stage I malignant melanoma can be divided into the control of the local lesion and the control of disease in draining lymph nodes.

Local Control

To achieve adequate local control of malignant melanoma, wide surgical excision is necessary. Patients who have "local" excisions of malignant melanoma with narrow margins have approximately an 80% chance of local recurrence. Adequate excision of malignant melanoma should adhere to two basic principles: (*a*) the area of the excision of the malignant melanoma should extend at least 4 cm in all directions from the lesion; and (*b*) closure of the excised wound should be managed by placement of a split-thickness skin graft.

Wide excision is absolutely essential for local control of melanoma. In the great majority of instances, adequate excision will necessitate the use of split-thickness skin grafts. As a general rule, any malignant melanoma excised without place-

TABLE 1.—Clinicopathologic staging of malignant melanoma

Stage	Description
I	Localized primary melanoma
IA	Local recurrence
II	Metastases to regional lymph nodes
III	Disseminated melanoma (visceral organs, distant lymph nodes, or distant cutaneous metastases)

ment of a skin graft has not been adequately excised. There is some debate as to whether the deep fascia should be included in the local excision specimen (2). Subungual melanomas require amputation of the involved digit, generally at the metatarsal or metacarpal-phalangeal joint. The treatment of melanomas occurring on the sole of the foot should conform to the above principles. Split-thickness skin grafts will generally tolerate weight-bearing and the fear of wound-breakdown in this area should not compromise adequate local excision.

Regional Lymph Nodes

The question of whether to dissect the regional lymph nodes in clinical stage I disease has been a controversial issue for many years. There is a vast literature arguing both sides of this question. In this review, I would like to present what I think is a logical approach to this problem.

The recent work of Clark et al (3) and others (4,5) has succeeded in identifying histologic characteristics important in the prognosis of patients with primary melanoma. Primary malignant melanomas are divided into five levels as presented in table 2. Progressively deeper invasion into the papillary dermis, the reticular dermis, and the subcutaneous fat is associated with a progressive decrease in survival. A study by McGovern (5) evaluating the 5-year survival of two morphologic types of malignant melanoma of different levels of invasion, is presented in table 3 and clearly demonstrates the poor prognosis associated with deep invasion.

Most reports of the correlation of histologic level with survival have combined clinical stage I and clinical stage II patients. It has recently become apparent, from the studies of Wanebo et al (6), that in patients with clinical stage I disease there is a highly significant correlation between the histologic level and the incidence of positive lymph nodes (table 4). Patients with level 2 or 3 clinical stage I malignant melanoma have an extremely low incidence of positive lymph nodes. Patients with level 4 or 5 melanoma have a 25% and 70% incidence of positive lymph nodes respectively. In clinical stage I patients regional lymph node dissection will, therefore, result in removal of tumor in significant numbers only in patients with level 4 or 5 primary lesions.

It also appears that the presence of positive nodes overrides the prognostic importance of the histologic level of the primary itself. Decreased survival in patients with increasing level of invasion appears to be due to the fact that the incidence of positive nodes is greater in those patients with deeper levels of invasion. This is also demonstrated in the studies of Wanebo et al (6) (table 5). Patients with level 4 disease and positive nodes had only a 27% 5-year survival compared to 82% for patients with negative nodes. It is clear therefore, that in clinical stage I patients, the presence or absence of microscopically positive nodes is a critical factor associated with prognosis.

As has been mentioned, there is much controversy as to whether prophylactic lymph node dissection affects survival. The only extensive prospec-

TABLE 2.—Level of invasion in malignant melanoma

Level	Description
1	All tumor cells above basement membrane
2	Invasion into loose connective tissue of papillary dermis
3	Tumor cells at junction of papillary and reticular dermis
4	Invasion into reticular dermis
5	Invasion into subcutaneous fat

TABLE 3.—Correlation of level of invasion of malignant melanoma and 5-yr survival [*from* McGovern (5)]

	5-Yr survival	
Level	Superficial spreading disease (%)	Nodular disease (%)
2	81	86
3	65	59
4	56	45
5	50	23

TABLE 4.—Relationship of level to incidence of positive nodes in patients with stage I melanoma of the extremities [*from* Wanebo et al (6)]

Level	No. of patients	% with positive nodes
2	28	4
3	62	7
4	69	25
5	10	70

TABLE 5.—Survival of patients with stage I melanoma of the extremities [*from* Wanebo et al (6)]

	5-Yr survival* (no evidence of disease) (%)	
Level	Positive nodes	Negative nodes
2	100 (1)	100 (18)
3	100 (2)	91 (44)
4	27 (11)	82 (33)
5	0 (3)	0 (1)

*No. of patients is in parentheses.

tive randomized study of this question has recently been reported by the International Group for Clinical Study of Melanoma (7). In this ongoing study the 3-year survival rate of patients having primary excision alone was compared with that of patients having local excision plus lymph node dissection. The preliminary results of this study are presented in table 6. As can be seen, the 3-year survival rates of these two groups are identical.

Regional lymph node dissection, especially in the inguinal area, is by no means a benign procedure and in some cases has a significant morbidity. Table 7 presents the major complications of lymph node dissection in the inguinal area, summarizing the results of many reported studies (1,8-10). Significant wound complications, including necrosis of skin flaps or infection, occur in 30%-40% of patients, and lymphedema occurs in 20%-30%. Hemorrhage from erosion into the femoral or iliac arteries or veins has also been reported in the postoperative period. It is hard to discern the exact incidence of pulmonary embolus following inguinal lymph node dissection. However, these patients are often elderly, requiring prolonged bed rest for healing of skin flaps, and pulmonary embolus in the postoperative period is a definite threat.

What, then, is the logical approach to the treatment of regional lymph nodes in clinical stage I disease? There are two possible reasons to consider removal of regional lymph nodes. These are (*a*) the possibility that a therapeutic effect may be obtained by removal of the positive nodes, and (*b*) identification of positive nodes may be useful in the selection of adjunctive therapy because of the poor prognosis of these patients.

It appears that lymph node dissection may be of no *therapeutic* help in the majority of patients with melanoma though this question is still unresolved. However, because of the extremely poor prognosis of patients with positive lymph nodes and the current availability of adjunctive measures such as chemotherapy and immunotherapy that may be of value in this poor prognosis population, it is of value to know which patients have positive lymph nodes.

TABLE 6.—Effect of lymph node dissection on survival of patients with melanoma [*from* Veronesi et al (7)]

	No. of patients alive at 3 yrs/ No. treated	3-Yr survival (%)
Excision plus lymph node dissection	120/140	85.7
Excision only	126/147	85.7

TABLE 7.—Complications of inguinal lymph node dissection

Complication	Occurrence (%)
Skin necrosis	30-40
Lymphedema	20-30
Infection	10-15
Hemorrhage	1-3
Pulmonary embolus	?

Regional lymph node dissection will identify those patients with positive nodes but will have significant yield only in patients with level 4 or 5 primary lesions. The incidence of positive lymph nodes is extremely small in level 2 and 3 primary lesions. Regional lymph node dissection is not justified for level 2 lesions and should probably not be performed in the inguinal region for level 3 lesions either, because of the morbidity of this procedure.

Before leaving the discussion of the treatment of stage I malignant melanoma, it should be pointed out that there are new approaches being tested in an attempt to improve the survival of patients with this disease. Experimental data from animal tumor systems suggest that the direct injection of bacillus Calmette-Guérin (BCG) into local lesions can be effective in destroying disease locally, as well as in draining lymph nodes. These systemic effects of intralesional BCG are achieved only when the primary nodule is injected and are not seen when *gross* distant disease is present. The application of these findings from experimental tumor systems has led to the development of a new clinical protocol now in progress at the NCI for the treatment of primary malignant melanoma (fig 1).

Level 4 or 5 lesions receive either:
1. Immediate standard surgery, or
2. BCG injection followed by standard surgery 2-3 weeks later.

FIGURE 1.—Current investigational protocol for the treatment of primary malignant melanoma at the NCI.

In this protocol, lesions suspected of being malignant melanomas are *partially* biopsied to determine the level of invasion. Level 4 and 5 lesions are then randomized to receive either surgery immediately (standard treatment) or injection with 6×10^8 viable BCG organisms. The local lesion and the regional lymph nodes are excised 2 weeks later in this second group.[2]

[2]The NCI is seeking patients with primary malignant melanoma with portions intact, or patients with skin lesions suspected of being malignant melanoma, for entrance into this protocol. For further information about referring suitable patients to the NCI, please contact Dr. Steven A. Rosenberg at the address shown on page 159 (area code 301 496-4164).

Stage IA

In the presence of a very small number of locally recurrent lesions, principles similar to those followed for the local control of primary melanoma should be adhered to. Wide local excision with skin grafting should be performed.

Some patients, however, may have multiple local cutaneous metastases at the time of diagnosis. In this situation local excision can often be fatal. Many studies have demonstrated that injection of BCG into cutaneous recurrences of malignant melanoma will result in the disappearance of 90% of injected lesions. In the presence of multiple cutaneous recurrences, the intralesional injection of BCG is a reasonable means of attempting to achieve local control. These patients have an extremely poor prognosis and the morbidity associated with massive resectional surgery is probably not justified, nor does it appear to offer better results. Up to 20% of non-injected melanoma lesions on the same extremity or in the same lymphatic drainage field may also regress. However, there is little chance that distant cutaneous recurrences out of the regional lymphatic drainage field will be affected. It should be noted that *subcutaneous* recurrences of malignant melanoma do not respond well to BCG injection and should be locally excised.

It is not clear that there is any increase in systemic resistance to malignant melanoma resulting from the injection of recurrent cutaneous nodules. Distant disease is unaffected as are cutaneous recurrences in other parts of the body. BCG injection of recurrent cutaneous nodules is suggested here solely as a means of achieving adequate local control without the morbidity of excessive surgery. Preliminary studies by Dr. Max H. Cohen in the Surgery Branch of the NCI have shown that it may be possible to use organic chemicals such as dinitrochlorobenzene (DNCB) as a substitute for BCG in the intralesional treatment of recurrent melanoma. DNCB may have some advantages over the use of live BCG organisms for this purpose.

STAGE II

Local control of patients with malignant melanoma with clinically positive lymph nodes is identical to that of patients with clinical stage I disease. All patients with clinically positive draining regional nodes and no evidence of distant metastatic spread should undergo regional lymph node dissection. All patients with documented disease in regional lymph nodes probably require some adjunctive therapy. It is thus essential to identify clinical stage II patients who truly have positive nodes.

TABLE 8.—Accuracy of clinical staging of lymph node status in melanoma [*from* Knutson et al (1)]

Clinical evaluation of lymph nodes	No. of patients	% of wrong decision (based on histologic diagnosis)
Positive	64	37.5
Negative	63	22.2

The false-positive rate for the clinical evaluation of melanoma in draining lymph nodes is high. As can be seen in the study of Knutson et al (1) (table 8), 37.5% of all patients who appeared to have clinically positive nodes on physical examination were found *not* to have lymph node involvement in histologic examination of regional lymph node dissection specimens. Therefore, it would not be justified to treat all patients with clinical stage II disease with adjunctive measures, and regional lymph node dissection is valuable in identifying those patients who truly have lymph node involvement. The removal of bulk tumor in involved nodes may also be of value for the success of adjunctive therapies.[3]

If the superficial inguinal lymph nodes are negative for tumor, then the deep inguinal lymph nodes will very rarely be positive and deep inguinal lymph node dissection is not justified. In the presence of clinically apparent tumor in the superficial inguinal lymph nodes, the deep nodes are more likely to be involved with tumor. About 10%-15% of patients with grossly positive superficial inguinal lymph nodes will be found to have simultaneous malignant melanoma in the para-aortic lymph nodes or liver if laparotomy is performed.

It is doubtful that the small additional tumor removed from patients with positive deep inguinal and iliac lymph nodes will affect the natural course of melanoma or the response to adjunctive treatment, but there are no conclusive data on this question.

STAGE III

The role of surgery in disseminated melanoma can only be considered in conjunction with the availability of other modalities for the control of metastatic disease. The effectiveness of suitable

[3]The role of immunotherapy in the treatment of malignant melanoma patients with positive lymph nodes is currently under evaluation at the NCI. For the referral of patients with positive lymph nodes or to obtain further information, please contact either Dr. William D. Terry, Chief, Immunology Branch, or Dr. Steven A. Rosenberg, Chief, Surgery Branch, at the address noted on page 159.

chemotherapy and immunotherapy for disseminated malignant melanoma may well be a function of the bulk of disease present at the time of treatment. If this is so, the surgical removal of bulk disease in the lung, liver, and other sites may well be a part of the *future* treatment of malignant melanoma. At the present time, there are no data indicating the usefulness of this "de-bulking." Anecdotal reports of the long-term survival of occasional patients who undergo pulmonary resection for single or small numbers of metastatic nodules have provided a stimulus for these limited pulmonary resections, both for their diagnostic value and for their possible therapeutic benefit.

CONCLUSION

The major lesson for the surgeon in the treatment of malignant melanoma is that, "Surgery is not enough." Of all patients who come to a surgeon with a primary malignant melanoma, most will ultimately die of that disease. Effective adjunctive therapy for most patients with malignant melanoma is a vital aspect of the treatment of this disease, and surgeons should be heavily involved in its development and administration.

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DTIC (NSC-45388) in Malignant Melanoma: A Perspective

Robert L. Comis[1,*]

The majority of patients with malignant melanoma die within 10 years of diagnosis with disseminated disease (1). Significant gains in overall survival will occur only with the development of effective systemic therapy which can eradicate either clinically established metastases or subclinical metastases present at the time of primary therapy. A large number of chemotherapeutic agents have been evaluated in this disease; DTIC has been the most extensively studied single agent. The purpose of this paper is to put into perspective the role of DTIC in disseminated melanoma within the context of existing data available for other agents.

The results presented in this paper are based upon a review of the published literature and data on file at the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute. The review will be confined to the systemic chemotherapy of disseminated disease. Complete response is defined as disappearance of all demonstrable disease; partial response represents a $> 50\%$ reduction of measurable lesions without evidence of the progression or appearance of any other lesion. The use of criteria less stringent than these is noted in the text.

SINGLE-AGENT CHEMOTHERAPY

"Standard" Agents

Several currently available commercial agents have been evaluated, but most of these studies were broad phase II trials not specifically designed for malignant melanoma. Many of them did not employ currently accepted criteria for defining objective response. In this review, an attempt has been made to separate the studies using a criterion of $\geq 50\%$ decrease in tumor from those where the decrease is $< 50\%$ or only general statements are given concerning objective decrease in the size of the lesions. If the more stringent parameter of response is used, few conventional agents have received adequate evaluation, but even so, in view of the total data, none has shown particularly striking activity (table 1).

None of the alkylating agents exhibit significant clinical activity. Cyclophosphamide has an overall response rate of 16%, but two recent studies (11,12) using the high intermittent dose schedule reported only one response out of 19 cases. Melphalan is currently the most widely used agent for isolated perfusion, yet its activity as a systemic agent is substantiated only by a few responses obtained in several older studies.

The antimetabolites have been consistently inactive against this disease. No agent in this class has yielded more than an 8% response rate, which is probably within the range of false positive error in measurement.

Actinomycin D has not been critically evaluated as a single agent, but it may have some activity. There are two grossly conflicting studies, neither of which defined objective response by currently accepted criteria. Golomb et al (29) found no responses in 23 consecutive patients, while Molander and Oropeza (30) reported a 67% response rate in 24 patients.

Vincristine has an overall response rate of 12% but, probably because of its nonmyelosuppressive qualities, it has been used extensively in combination regimens. Vinblastine has a similarly low order of activity.

The initially high response rates cited for hydroxyurea have not been substantiated by more critical analysis and several recent studies show only nominal activity (13,46,49,56-58). Procarbazine may have some activity in this disease, although it has never had an adequate and critical evaluation.

Imidazole Carboxamides

DTIC has been the most extensively studied single agent in malignant melanoma. The toxic effects of this drug are well known and consist of moderate leukopenia (white blood cell count < 3000 cells/mm^3) and thrombocytopenia (platelet count $< 100{,}000$ cells/mm^3) in approximately one quarter of the patients, moderate to severe nausea and vomiting in the majority of patients, occasional hepatic dysfunction, and a flu-like syndrome consisting of fever, myalgias, and malaise. From table 2 it can be seen that a variety of doses and schedules have been employed clinically, and it appears that, as in the L1210 system, the therapeutic effects are independent of schedule.

The Eastern Cooperative Oncology Group (ECOG) (64) compared a 10-day schedule of DTIC at 4.5 and

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TABLE 1.—Activity of "standard" agents against disseminated melanoma

Antitumor agent	Objective response: Vague* No. of responses/ No. of evaluable patients	Ref No.	Objective response: >50% decrease in measurable disease No. of responses/ No. of evaluable patients	Ref No.	Overall response
Alkylating agents					
Mechlorethamine	3/45(7%)	2,3	—		3/45(7%)
Cyclophosphamide	8/36(22%)	4-10	1/19(5%)	11-13	9/55(16%)
Melphalan	8/49(20%)	14-16†	0/3	13	8/52(15%)
Chlorambucil	—		2/22(9%)	17	2/22(9%)
Antimetabolites					
6-Mercaptopurine	0/13	18	2/34(6%)	19-21	2/47(4%)
5-Fluorouracil	0/24	22	1/19(5%)	13,23	1/43(2%)
Methotrexate	0/5	24	2/20(10%)	13,22,25	2/25(8%)
Cytosine arabinoside	—		1/52(2%)	22,26-28	1/52(2%)
Antibiotics					
Actinomycin D	19/55(35%)	29-31	—		19/55(35%)
Mithramycin	9/79(11%)	32	—		9/79(11%)
Vinca alkaloids					
Vincristine	3/33(9%)	33,34	3/19(16%)	35-38	6/52(12%)
Vinblastine	6/32(19%)	39-43	5/39(13%)	44-50	11/71(15%)
Miscellaneous					
Hydroxyurea	23/59(39%)	51-55	6/68(8%)	13,46,49, 56-58	29/127(23%)
Procarbazine	4/40(10%)	59,60	4/10(40%)	46,61	8/50(16%)

*< 50% decrease in measurable disease or inadequate criteria of objective response.

†Buce F. Data on file, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

TABLE 2.—Activity of DTIC against malignant melanoma

Investigator and ref No.	DTIC dose schedule*	No. of evaluable patients	CR + PR No.	CR + PR Overall %
Wagner et al (62)	4.5 mg/kg/day × 10	393	109	28
Luce (63)	250 mg/m²/day × 5	125	20	16
Nathanson et al (64)	2 or 4.5 mg/kg/day × 10	115	32	28
Costanza et al (65)	150 mg/m²/day × 5	51	9	31
Moon et al (66)	300 mg/m²/day × 6 or 100 mg/m² q8h × 6	46	12	27
Falkson et al (67)	100 mg/m² q8h × 6 or 150 mg/m²/day × 10 or 300 mg/m²/day × 6	29	8	28
Gottlieb et al (68)	150 mg/m²/day × 5 or 350-450 mg/m² biweekly	25	3	12
Burke et al (69)	4.5 mg/kg/day × 10	20	4	20
Cowan et al (70)	650-1450 mg/m² q4-6wk	20	4	20
Gerner et al (71)	3.5-5.5 mg/kg/day × 10	15	3	20
Vogel et al (72)	300-400 mg/m²/day × 5	12	1	8
Totals		851	205	24

*All studies used the iv formulation of the drug.

TABLE 3.—DTIC: survival from treatment and response duration

Investigator and ref No.	No. of evaluable patients	Survival from treatment (mos)		Response duration (mos)
		Responders	Nonresponders	
Luce (63)	125	11.3*	3.6*	5.7*
Nathanson et al (64)	115	10†,‡	2.8†,‡	6.7†,‡
Costanza et al (65)	51	6.5*,‡	2.8*,‡	5.0*,‡

*Median.
†Mean.
‡When data were presented in days or wks, a conversion was made to mos.

2.0 mg/kg. The response rate was actually higher with the lower dose (33% vs 22%) and was significant at the $P < 0.05$ level. The two groups were said to be comparable in several important factors, such as sex, prior chemotherapy, and site of metastasis, although no detailed analysis of these factors was presented. There was a trend toward less severe myelosuppression in the low-dose group and it was suggested that the possible beneficial effect of the lower dose might be related to less severe immunosuppression. The Acute Leukemia Group B (ALGB) compared 300 mg/m²/day × 6 to 100 mg/m² q8h × 6 and found no difference in response rate (24% vs 29% respectively) (66). There were two drug-related deaths from severe thrombocytopenia and associated brain hemorrhage on the q8h schedule. A variety of other schedules have been used in largely uncontrolled studies and there does not appear to be any definite schedule or dose-dependent relationship to therapeutic effect.

Since the publication of the last large review article (73) that showed a 21% response rate in 426 patients, the response data from the literature have remained essentially unchanged with 205 responses in 851 patients (24%). Most of the data are from five large cooperative group studies (62-66) and the response rate in each is in the rather narrow range of 16%-31%. Complete response rates of 5%-6% have been consistently observed in each of the large studies, with an overall complete remission rate of 5% (38 of 730 patients). It must be emphasized that in virtually all studies there are consistent trends in responders versus nonresponders. The responders tend to be previously untreated, women, with soft tissue, nodal, and skin disease. The most resistant sites of involvement are bone, liver, and brain with lung being intermediate between these sites and the responsive sites mentioned previously.

In the reports on all major studies, responders have lived longer than nonresponders, as might be expected. Data are available on the duration of survival from the start of therapy and duration of response in three major studies (table 3). Although it is impossible to tell from these data whether the increase in survival from the start of therapy represents patient selection factors or an actual effect of the drug, there does appear to be a rough correlation between the duration of response and the survival gain of the responders.

A detailed analysis of survival in the large Southwest Oncology Group (SWOG) study is presented in table 4. The number of 1-year survivors is five times greater for responders than for nonresponders, and as previously mentioned, women fare better than men not only in survival from start of treatment but also in response rate.

BIC, an analog of DTIC, is more active than the parent compound in the L1210 leukemia system given on a daily short-term or a high intermittent dose schedule. Data are available on the 112 patients showing an overall response rate of 8% (74,75).[2] A direct comparison of BIC and DTIC recently completed by the ECOG shows statistically significant superiority for DTIC in the treatment of disseminated malignant melanoma.

Nitrosoureas

In view of the propensity of malignant melanoma to metastasize to the brain, the agents in this class of antitumor compounds are of particular interest because of their relatively high lipid solubility and ability to cross the blood-brain barrier.

The first-generation nitrosourea, BCNU, has undergone extensive clinical trials. Published data, relating only to the objective response rate, are currently available for 122 patients showing a response rate of 18% (table 5).

CCNU, the first oral congener of BCNU, has an objective response rate of 13% in 133 patients with disseminated malignant melanoma. One complete response was noted in a broad phase II study reported by Hoogstraten et al (82). Two studies were disease-oriented randomized studies of CCNU as pri-

[2]Costanza M. Personal communication.

TABLE 4.—Survival from start of chemotherapy with DTIC (data from ref 63)

		Median survival (mos)
No. of patients treated	126	4.6
No. of responders	20	11.3
Women	14	13.7
Men	6	7.3
No. of nonresponders	106	3.6
1-yr survival		
Responders	50%	
Nonresponders	9.5%	

mary therapy compared to combination chemotherapy (79, 81). In these studies, the drug yielded disappointingly low response rates of 4% and 5%. In comparing CCNU to CCNU plus vincristine, the Western Cancer Study Group (79) found a low response rate for both regimens but a statistically significant superiority for the combination versus CCNU. When CCNU was compared to DTIC plus vincristine at the Mayo Clinic (81), the response rate with the single agent was lower than that with the combination, but the difference was not statistically significant. In the latter study, the majority of patients had visceral dominant disease.

Methyl-CCNU, which is the newest nitrosourea to enter clinical trials, is the most highly lipid soluble of the three nitrosoureas currently undergoing clinical evaluation. It is also the most effective agent available in the Lewis lung tumor system, a low growth fraction solid tumor in mice. The drug has been evaluated in five studies, three of which were designed specifically for malignant melanoma. The Mayo Clinic study (87) compared methyl-CCNU to a combination of DTIC plus vincristine in a randomized crossover design. Five responses were noted among 28 patients, all of which occurred in patients who had not had prior therapy and none of which occurred in patients who had failed therapy with the combination. There was no difference in response between the combination and the single agent. The National Cancer Institute (89) has completed a trial of 28 consecutive patients with disseminated malignant melanoma, five of whom had prior chemotherapy. Twenty-one patients received doses of methyl-CCNU of 250 mg/m^2, while seven received doses ranging from 40 to 170 mg/m^2. Responses ranging from 2 to 18 months were seen in six patients. Interestingly, five of 19 men and one of nine women responded; one complete response was noted. Regression of visceral (lung) as well as nonvisceral disease was found in four of six patients.

TABLE 5.—Activity of the nitrosoureas against malignant melanoma

Investigator and ref No.	Dose schedule	No. of evaluable patients	Response	
			No.	Overall % (CR+PR)
BCNU				
Ramirez et al (76)	1.5 mg/kg/day × 5	99	19	19
DeVita et al (77)	150 mg/m^2/day × 3	20	3	15
Lessner (78)	100 mg/m^2/day × 2-3	3	0	0
		122	22	18
CCNU				
Pugh et al (79)	S*	48	2	4
Cruz et al (80)	S	27	7	26
Ahmann et al (81)	S	19	1	5
Hoogstraten et al (82)	100 or 130 mg/m^2 q6wk	14	3	21
Solomon et al†	S	12	2	17
Broder et al (83)	130 mg/m^2 q4-6wk	4	0	0
DeConti et al (84)	S	4	1	25
Perloff et al (85)	130 mg/m^2 q4-6wk	3	1	33
Hansen et al (86)	100-130 mg/m^2 q6wk	2	0	0
		133	17	13
Methyl-CCNU				
Ahmann et al (87)	225 mg/m^2 q6wk	28	5	18
Costanza et al (88)	200 mg/m^2 q6wk	37	7	19
Young et al (89)	220 mg/m^2 q6wk	28	6	21
Tranum et al (90)	150 or 200 mg/m^2 q6wk	10	0	0
Firat et al (91)	200 mg/m^2 q6wk	5	0	0
		108	18	17

*S = standard dose CCNU (130 mg/m^2 orally q6wk).
†Solomon J, and Bateman J. Personal communication.

TABLE 6.—Activity of other investigational agents in malignant melanoma

Drug	Ref No.	No. of evaluable patients	Response	
			No.	%
Pregnenetrione	92,93	155	11	7
Trimethylcolchicinic acid	94,95	58	7	12
Mitomycin C	96-98	65	9	14
Hexamethylmelamine	99	42	2	5
Bleomycin	100	34	0	0
Adriamycin	101	23	3	13
Dibromodulcitol	102,103	18	3	17
5-Azacytidine	104,105	16	3	19

TABLE 7.—Activity of 2-drug combination regimens against malignant melanoma

Investigator and ref No.	Regimen	No. of evaluable patients	Response	
			No.	% (CR+PR)
Combinations with DTIC				
Gerner et al (106)	DTIC (2.5 mg/kg/day × 10 q4wk) Actinomycin D (0.25 mg/day × 10 q4wk)	69	14	20
Einhorn et al (107)	DTIC (100 mg/m²/day × 5) Procarbazine (100 mg/m²/day × 10)	62	10	16
Costanza et al (65)	DTIC (100 mg/m²/day × 5) BCNU (75 mg/m²/day × 2)	61	12	19
Ahmann et al (81,87)	DTIC (315 mg/m²/day × 5) Vincristine (1.4 mg/m² Days 1 and 5)	56	9	16
Costanza et al (88)	DTIC (150 mg/m²/day × 5) Methyl-CCNU (130 mg/m²/day × 1)	30	6	20
Combinations with nitrosoureas				
Moon et al (66)	BCNU (150 mg/m²/mo) Vincristine (2 mg/m²/mo)	51	8	16
Moon (108)	BCNU (150 mg/m²/mo) Vincristine (150 mg/m²/mo)	20	9	45
Marsh et al (109)	BCNU (150 mg/m²/mo) Vincristine (2 mg/m²/mo)	11	2	18
Pugh et al (79)	CCNU (130 mg/m² q6wk) Vincristine (1 mg/m² q3wk)	41	8	20
Other combinations				
Chanes et al (110)	Actinomycin D (10 μg/kg/day × 5; then after 2 wk, qwk × 4; then qowk) Vincristine (25 μg/kg/wk × 4; then qowk)	12	6	50
Johnson et al (111)	Cyclophosphamide (2 mg/kg/day × 28) Vincristine (25 μg/kg/wk)	9	0	0

No responses were observed in 15 patients treated in broad phase II studies. There are no details of the extent of disease, sex, or prior therapy of these patients. Methyl-CCNU appears to be active as a first-line drug against melanoma. Importantly, however, responses have not been seen after crossover to other regimens including DTIC. Whether this represents true drug cross resistance or the refractoriness of more advanced disease is unknown.

There is currently no conclusive evidence that the nitrosoureas used alone or in combination enhance the response in central nervous system metastasis or delay the development of central nervous system involvement (65,107).

Other Antitumor Agents

A variety of other single agents have been investigated in this disease with only a few responses (table 6). Bleomycin, a drug that localizes in the skin, has thus far been totally inactive. Adriamycin has a broad spectrum of activity in solid tumors, but has minimal activity in melanoma. Dibromodulcitol gave a hint of activity in one phase I study and is of particular interest because it has been shown to cross the blood-brain barrier. Initial reports of activity for pregnenetrione and trimethylcolchicinic acid were never substantiated in larger studies.

COMBINATION CHEMOTHERAPY

As shown above, a variety of single agents have been tested in disseminated melanoma and many of them exhibit modest activity. The use of these agents in two-, three-, four-, and five-drug combinations, with special emphasis on those employing DTIC and/or the nitrosoureas, is reviewed below. This imparts an artificial order to the chaotic development of combinations that in no way corresponds to the chronologic events in the development of multidrug regimens for this disease.

Five two-drug combinations have employed DTIC plus another drug (table 7) but the response rate for each is apparently no different from that obtained with DTIC alone. Two of the five studies, both performed by the ECOG (65,88), have included DTIC given in equitoxic doses as a control arm. An extensive analysis of the BCNU plus DTIC study showed no difference in response rate, duration of response, overall survival from start of chemotherapy, or responsiveness of various metastatic sites. Ahmann's studies (81,87) employing relatively high doses of DTIC have yielded about a 20% objective response rate in previously untreated patients with predominantly visceral disease.

Moon's original study (108) reporting a high response rate for the combination of BCNU and vincristine was not substantiated by a large controlled study of the ALGB (66) that compared the combination to two dose schedules of DTIC. Overall, the studies show a 20% response rate in 82 patients treated with BCNU and vincristine.

Two additional two-drug combinations not employing DTIC or a nitrosourea have been reported in the literature. The study of Chanes et al (110) used a 25% decrease in the sum of the perpendicular diameters of measurable disease as an objective response, which may account for the high response rate in a few patients.

Table 8 shows the results with three-drug combinations. DTIC, BCNU, and vincristine (BVD) have been combined in four separate studies. The SWOG sequentially studied two different doses of the combination without obtaining an apparent difference in response rates. Currently, the study is undergoing definitive analysis and it is not known whether there is a difference in duration of response or an increase in the responsiveness of visceral disease.

Einhorn et al (107) has recently reviewed the M.D. Anderson Hospital experience in three sequential studies conducted from 1967 to 1972 with regimens of DTIC alone, DTIC plus procarbazine, and BVD. These studies are not included in the tables since some of the patients (those receiving DTIC alone and the BVD combination) were entered in the SWOG studies. The overall response rates, duration of response, and survival were not significantly different. An analysis of the patterns of response show a higher proportion of responses in visceral disease with the three-drug combination compared to DTIC alone (17 of 20 vs ten of 19). Patient numbers and inadequate analysis make it impossible to state whether this has also been the experience in the 198 patients receiving BVD noted in the table.

Three of the four BVD studies used comparable doses and schedules, whereas the study recently reported by Cohen et al (114) employed somewhat more intensive courses of DTIC and BCNU. This preliminary report showed responses in ten of 16 patients, with most responses being in nodal and subcutaneous disease. The more intensive approach has not been evaluated in a controlled fashion, and it is difficult to assess whether patient selection factors or the higher doses contributed the critical variable in this small study.

A report on the combination of DTIC, BCNU, and hydroxyurea (BHD) showed a response rate of 38% (24 of 64) in evaluable patients (115). Of the eligible patients, 30% were considered early deaths (< 28 days). Most of these patients had liver and brain metastases, which are two of the most common and

TABLE 8.—Activity of 3-drug combination regimens against malignant melanoma

Investigator and ref No.	Regimen	No. of evaluable patients	Response	
			No.	%
Luce et al (112)*	DTIC (100 or 150 mg/m²/day × 5) BCNU (100 or 150 mg/m²/day × 1) Vincristine (1.4 mg/m² Days 1 and 4)	79	18	23
Luce*	DTIC (30 mg/m² Day 1; then 80 or 120 mg/m²/day × 4) BCNU (80 or 120 mg/m²/day × 1) Vincristine (1.5 mg/m² Days 1 and 4)	62	18	29
Beretta et al (113)	DTIC (100 mg/m²/day × 5) BCNU (100 mg/m²/day × 1) Vincristine (1.4 mg/m² Days 1 and 4)	41	8	20
Cohen et al (114)	DTIC (400 mg/day × 3-4) BCNU (100 mg/day × 3-4) Vincristine (1-1.5 mg/wk)	16	10	63
Costanzi et al (115)	DTIC (100 or 150 mg/m²/day × 5 q3wk) BCNU (100 or 150 mg/m²/day × 5 q6wk) Hydroxyurea (1480 mg/m²/day × 5 q3wk)	64	24	33
Carter et al (116)	DTIC (150 mg/m²/day × 5) CCNU (50-80 mg/m²/day × 1) Vincristine (0.7-1.4 mg/m² Days 1 and 5)	11	4	36
Gardere et al (117)	DTIC (850 mg/m²/day × 1) Cyclophosphamide (750 mg/m²/day × 1) Vincristine (2 mg/m² q4wk)	20	5	25
Nathanson et al (118)	Mitomycin C (0.1 mg/kg/wk) Melphalan (0.2 mg/kg/wk) Vincristine (0.025 mg/kg/wk)	12	1	8

*Luce JK. Personal communication.

resistant visceral sites. As with single-agent DTIC, responses were seen primarily in patients with skin, nodal, or lung metastasis; when all 89 patients are included in the denominator, the response rate is 27%.

The findings with the four- and five-drug combinations are presented in table 9. The SWOG added vincristine to their BHD combination, comparing it to the three drugs. The response rates were similar as was the high proportion of early deaths. The rest of the combinations did not include DTIC, but there appears to be some activity for these intensive combination regimens.

DISCUSSION

The control of disseminated malignant melanoma presents formidable problems. It is one of the few diseases in which the majority of clinically active chemotherapeutic agents have been tested. None has shown activity > 25%. DTIC is the most extensively studied and consistently active single agent. Indeed, it is rare to observe such a narrow range of activity (16%-31%) in several large studies. On the other hand, most responses have occurred in nonvisceral disease (ie, skin, nodes, and soft tissue) and have been of relatively short duration. Responses in major visceral sites such as liver, brain, and lung are infrequent and inconsistent. As a class of compounds, the nitrosoureas have had an adequate evaluation. There does not appear to be any obvious superiority of one nitrosourea over the other. The degree of activity, both qualitative and quantitative, appears to be similar to DTIC although the evaluation of each drug has been less extensive. The crossover studies performed by Ahmann et al (81,87) are somewhat discouraging in that secondary therapy with either of the two most consistently active drugs, DTIC or a nitrosourea, yields no responses after failure on the alternate regimen. Therefore, there seems to be a small subset of all patients with melanoma who will initially respond to drugs of

TABLE 9.—Activity of 4- and 5-drug combination regimens against malignant melanoma

Investigator and ref No.	Regimen	No. of evaluable patients	Response	
			No.	%
Costanzi et al (115)	DTIC (100 or 150 mg/m²/day × 5 q3wk) BCNU (100 or 150 mg/m²/day × 1 q6wk) Hydroxyurea (1480 mg/m²/day × 5 q3wk) Vincristine (1.4 mg/m² Days 1 and 5 q3wk)	68	27	40
Costanzi et al (119)	Cyclophosphamide (300 mg/m² Days 1 and 5) Vincristine (0.025 mg/kg Days 2 and 5) Methotrexate (0.5 mg Days 1 and 4) 5-FU (10 mg/kg/day × 5)	10	6	60
Coltman et al (120)	Cyclophosphamide (200-300 mg Days 1 and 5) Vincristine (0.015 mg/kg Days 2 and 5) Methotrexate (0.25 mg/kg Day 1) 5-FU (7.5 mg/kg/day × 5)	7	2	28
Hanham et al (121)	Cyclophosphamide (200-300 mg Days 1 and 5) Vincristine (0.015 mg/kg Days 2 and 5) Methotrexate (0.25 mg/kg Day 1) 5-FU (7.5 mg/kg/day × 5)	10	0	0
Ramirez*	Cyclophosphamide (2 mg/kg/day) Vincristine (0.02 mg/kg/wk) Methotrexate (0.5 mg/kg/wk) 5-FU (10 mg/kg/wk) Prednisone (45 mg/day × 2 wk; then 30 mg/day × 2 wk; then 15 mg/day)	23	5	22

*Ramirez G. Personal communication.

modest activity. It might be anticipated that combinations of drugs would either increase the proportion of responding patients or the quality of response. Several different drug combinations have been reported in the literature, most of them including DTIC combined with other drugs.

Three studies, all employing a nitrosourea, compared a combination against DTIC alone and none has shown superiority for the combination. Most other studies have been uncontrolled and there is no conclusive evidence that combination chemotherapy enhances response rate, duration of response, or survival. The analysis of the M.D. Anderson experience by Einhorn et al (103), which indicates an increased number of visceral disease responses with BVD, may be important if substantiated after a thorough analysis of the SWOG studies and the controlled studies which are now in progress. The current data do not appear to support the use of multidrug approaches strongly enough to subject patients to the added toxicity of the combination of regimens thus far tested.

Currently, there are several studies employing adjuvant chemotherapy for melanoma patients at high risk for recurrence. The results of these studies should soon be forthcoming. Hopefully, systemic chemotherapy will show some effectiveness in the control of subclinical disease. There is still a significant need for developing and evaluating new drugs in malignant melanoma which might show greater clinical activity and be more effectively employed in combination chemotherapy and ultimately combined modality regimens. Also, the clinical as well as biochemical, pharmacologic, cytokinetic, and immunologic parameters that differentiate responders from nonresponders must be intensively studied. This may not only facilitate the prediction of response, but also could conceivably aid in the design of more rational chemotherapeutic approaches. Although malignant melanoma remains a difficult disease, the possibility exists that the development of effective systemic chemotherapy will alter the prognosis for the majority of melanoma patients.

LIST OF COMPOUNDS

Actinomycin D:NSC-3053
Adriamycin:NSC-123127; CAS reg. No. 23214-92-8
5-Azacytidine:NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

BIC: NSC-82196; CAS reg. No. 5034-77-5; imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1-6-dibromo-1,6-dideoxy-

DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Mithramycin: NSC-24559; CAS reg. No. 18378-89-7

Mitomycin C: NSC-26980; CAS reg. No. 50-07-7

Pregnenetrione: NSC-17256; CAS reg. No. 3642-85-1; pregn-4-ene-3,11,20-trione, 6α-methyl-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Trimethylcolchicinic acid: NSC-36354; colchicine, *N*-deacetyl-, L-tartrate (1:1), hydrate

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Bacillus Calmette-Guérin Immunotherapy in Combination With DTIC (NSC-45388) for the Treatment of Malignant Melanoma[1,2]

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SUMMARY

Combination studies of immunotherapy and DTIC chemotherapy for patients with malignant melanoma are described. At our institute the combination of bacillus Calmette-Guérin (BCG) with DTIC, or DTIC plus methyl-CCNU, has resulted in augmentation of remissions in areas regional to BCG immunization, prolongation of chemotherapy-induced remissions, and significant prolongation of survival compared to the use of DTIC chemotherapy without immunotherapy. Data from other centers have shown that augmentation of remissions for melanoma patients occurred when BCG was combined with DTIC, vincristine, and allogeneic tumor cells. Finally, another study has shown that DTIC combined with BCG has prolonged the disease-free interval and survival in melanoma patients with regional lymph node metastases. The potential inhibition of DTIC metabolism by BCG or *Corynebacterium parvum* is discussed.

[Cancer Treat Rep 60:177-182, 1976]

Since early in the 20th century, there has been evidence suggesting that immunologic manipulation of tumors in patients would slow tumor growth or cause regression of established malignancies (1). Although immunotherapy is therefore not a new medical discipline, it is only recently that results from comparative trials have established this therapeutic modality as a major new approach to cancer control.

In April 1972 we initiated our first trial of immunochemotherapy for a disseminated tumor: malignant melanoma. The major objectives of this study were to determine *(a)* if immunotherapy combined with chemotherapy could increase remission rates; *(b)* if immunotherapy could prolong chemotherapy-induced remissions; and *(c)* if chemoimmunotherapy could prolong the overall survival of melanoma patients compared to chemotherapy alone.

The first study of chemoimmunotherapy for disseminated melanoma with bacillus Calmette-Guérin (BCG) was published in 1974 (2). A chemotherapeutic drug, DTIC, was combined with large doses of fresh Pasteur BCG given by scarification in patients with disseminated melanoma. The major results were: *(a)* remission rates regional to sites of BCG administration were increased; *(b)* remissions were prolonged with chemoimmunotherapy compared to chemotherapy used alone; and *(c)* the overall survival of patients receiving chemoimmunotherapy was significantly prolonged compared to the survival of patients receiving chemotherapy alone (2).

Following these results, we designed a study exploring the question of whether methyl-CCNU[4] when added to DTIC plus BCG could improve the preceding results. The results of our study as well as those of other studies which combined immunotherapy with DTIC chemotherapy will be reviewed.

MATERIALS AND METHODS

Patients with disseminated metastatic melanoma (stage IV) were entered in the study between July

[1]Supported by contract N01-CB-33888 and Public Health Service grant CA-05831 from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health, Education, and Welfare (DHEW). Drs. Gutterman and Mavligit are the recipients of Career Development Awards (CA-71007 and CA-00130, respectively) from the NCI, NIH, DHEW.

[2]DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

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[4]Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

1973 and April 1974. Before chemoimmunotherapy, patients were evaluated for metastatic disease with the following tests: physical examination, complete blood cell counts, urinalysis, tests of liver and renal function, chest roentgenogram, liver scan, electroencephalogram, brain scan, and bone marrow biopsy. When specific symptoms were indicated, a metastatic bone survey, or a roentgenographic examination of the gastrointestinal tract, or both, were done.

The experimental design of the chemoimmunotherapy was as follows: the chemotherapy regimen included 250 mg/m^2 of DTIC given iv on Days 1-5 and 100 mg/m^2 of methyl-CCNU given orally on Day 1. Lyophilized Tice strain BCG in a dose of 6 $\times$ 10^8 viable units was given by scarification in the upper arms and upper legs on Days 7, 12, and 17 of each course (2). Courses were repeated every 21 days if the blood cell counts permitted.

If myelosuppression did not occur during the initial course, the DTIC-methyl-CCNU dosage was increased by 25% during the subsequent course. If the white blood cell (wbc) count was reduced to <1000 cells/mm^3, the granulocyte count to <500 cells/mm^3, or the platelet count to <50,000 platelets/mm^3, the DTIC and methyl-CCNU doses were reduced by 25%-50% during the next course. No adjustment was made if blood cell counts fell to the following levels: wbc, 1000-3000 cells/mm^3; granulocyte count, 500-1500 cells/mm^3; platelet count, 50,000-150,000 platelets/mm^3.

The following definitions were used: complete remission, complete disappearance of all objective and subjective disease manifestations; partial remission, ≥50% reduction in the area of all measurable tumor; stabilization, <50% reduction or <25% increase in tumor size for at least 2 months; progression, ≥25% increase in tumor masses or appearance of new masses; and relapse, reappearance of a tumor mass.

Survival was measured from onset of the treatment to death or date of last followup examination. The DTIC-methyl-CCNU-BCG (DMB) combination continued for a minimum of two courses unless the patient died. Therapy was stopped only on evidence of disease progression.

The results were compared to those obtained in the previous trial with DTIC-BCG and studies of DTIC used alone or in combination with the chemotherapeutic agents (*see* reference No. 2 for details).

The statistical methods used included a generalized Wilcoxon test and a one-tailed analysis for testing differences between remission or survival curves (3), and the methods of Kaplan and Meier (4) for calculating and plotting remission and survival curves.

RESULTS

The results of the DTIC-BCG combination study have been published elsewhere (2). Table 1 illustrates response rates in the DMB combination study. The overall remission rate was 27%. The only major difference in this study compared to the study with the DTIC-BCG combination was that half of the remissions were complete—for an overall complete remission rate of 13.5%. The percentages of stability and progression were approximately the same as noted in the previous study with DTIC-BCG.

Figure 1 illustrates the durations of remission for patients receiving DMB compared to those receiving DTIC and DTIC-BCG. Both groups of patients treated with BCG plus chemotherapy had significantly longer durations of remission than the group of patients treated with DTIC alone.

Figure 2 shows the overall survival of patients treated with DMB compared to the three previous studies with DTIC chemotherapy plus DTIC-BCG. The survival for both groups of patients treated with BCG and chemotherapy (DTIC + BCG and DMB) was significantly longer than the survival observed for patients in three previous studies

TABLE 1.—Chemoimmunotherapy of disseminated melanoma

Treatment regimen	No. of evaluable patients	% of patients with—		
		Remission	Stable disease	Progression
DTIC-BCG	89	27*	32	41
DMB	66	27†	40	33
DTIC-vincristine-BCG + tumor cells‡	30	55	—	—

*CR = 5%; PR = 22%.
†CR = 13.0%; PR = 13.0%.
‡*From* ref 5.

TABLE 2.—DTIC and immunotherapy for malignant melanoma

Therapeutic regimen	Extent of disease	Major therapeutic results	Ref No.
DTIC-BCG	Disseminated	Prolonged remission and survival	2
DMB	Disseminated	Prolonged remission and survival	*
DTIC-vincristine-BCG + tumor cells	Disseminated	Increased remission rate	5
DTIC-BCG	Disseminated	Prolonged survival	6
DTIC-BCG	Regional	Prolonged disease-free interval and survival	6

*Data from this study.

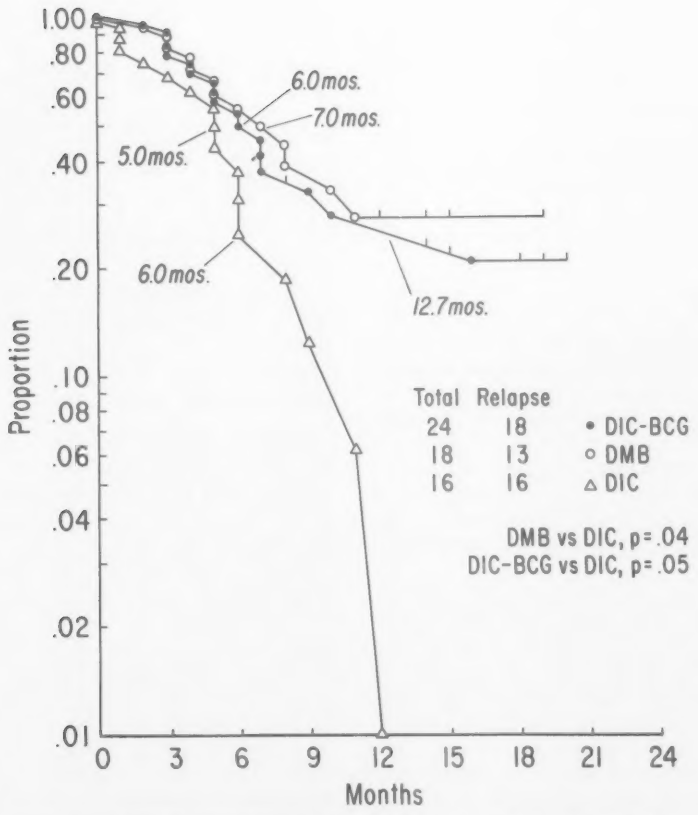

FIGURE 1.—Chemoimmunotherapy of disseminated malignant melanoma: duration of remission.

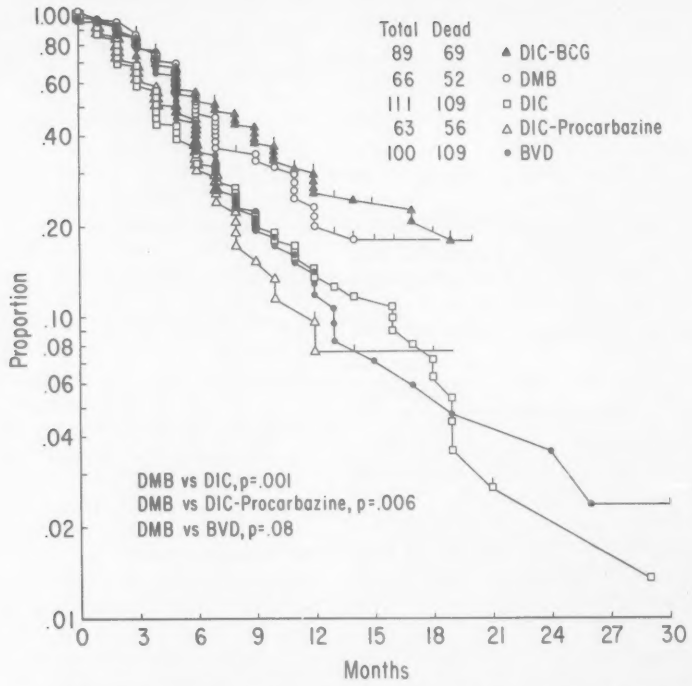

FIGURE 2.—Chemoimmunotherapy of disseminated malignant melanoma: survival. BVD = BCNU, vincristine, and DTIC.

with chemotherapy alone (DTIC, DTIC-procarbazine, and BCNU, vincristine, and DTIC).

We have initiated two subsequent immunochemotherapy studies with the DMB combination. The first study is evaluating irradiated allogeneic tumor cells plus the DMB combination. In an attempt to augment remission rates in visceral areas, a second study adding iv *Corynebacterium parvum* (*C. parvum*) to DMB has been initiated (see Discussion section). The results of these studies will be described in a subsequent report.

A summary of European trials demonstrating the efficacy of immunotherapy combined with DTIC is shown in table 2.

Currie and McElwain (5) demonstrated that a combination of DTIC, vincristine, and BCG, plus irradiated allogeneic tumor cells, resulted in an overall remission rate of 55% in a group of 25 consecutive patients with widespread disseminated melanoma. Secondly, Ikonopisov (6) has reported that BCG combined with DTIC has prolonged the postoperative disease-free interval and survival of melanoma patients with regional lymph node metastases. In addition, melanoma patients with disseminated disease had a better survival with BCG plus DTIC compared to DTIC alone. In all these studies the use of immunotherapy in combination with chemotherapy has been associated with minimal toxicity and no systemic BCG disease has been reported.

DISCUSSION

Although DTIC is the single most active drug for the treatment of malignant melanoma (7), the remission rates for chemotherapy used alone continue to be low. In addition, chemotherapy-induced remission durations and survival are short (2).

Combination studies of immunotherapy and chemotherapy have begun to yield interesting data. Active immunotherapy with BCG combined with DTIC has resulted in an overall increased remission rate in areas regional to the BCG vaccination. These results suggest that immunotherapy has the capacity to potentiate the chemotherapeutic action of a drug by directly activating components of the immune system which reside within tumor-involved areas. This concept is supported by experimental results with two human tumors, choriocarcinoma and Burkitt's lymphoma. In both of

these tumors, there is a vigorous host response to the tumor. Additionally, these tumors are exquisitely sensitive to chemotherapeutic agents, resulting in significant cures with relatively low doses of drug (8,9).

It is now known that melanomas are frequently infiltrated with a variety of cells, including macrophages and lymphocytes (10,11). It appears that if one can activate these cells by "regional immunotherapy" an additive or synergistic effect with chemotherapy may be achieved.

The capacity of immunotherapy to increase remission rates in disseminated melanoma has been confirmed by Currie and McElwain (5) who showed that allogeneic melanoma cells plus BCG combined with DTIC and vincristine lead to a remission rate of 55%. It is known that immunization with tumor cells decreases blocking factors in the serum possibly by increasing antitumor antibodies.

The major limiting feature thus far in the use of immunotherapy with DTIC for melanoma has been the low remission rates for patients with visceral metastases. However, immunotherapeutic approaches are available which may improve this situation. Specifically, the use of an iv immunopotentiator has the capacity to activate immune-derived cells (eg, macrophages) in the liver and lung. *C. parvum* or BCG delivered by the iv route is known to be highly effective for animals bearing pulmonary metastases (12,13). We have shown that iv *C. parvum* can be given safely and repeatedly to patients with far-advanced cancer; immunopotentiation occurs at doses of 5 mg/m^2 or greater (14). Thus, we are now exploring the use of iv *C. parvum* in combination with DTIC in patients with advanced melanoma to test the hypothesis of potentiating chemotherapeutic response in visceral regions.

DTIC appears to be an ideal drug to combine with immunotherapy. In contrast to most other chemotherapeutic agents, it is minimally immunosuppressive in man (15). In addition, DTIC has been shown to augment immunogenicity of tumor cells in various experimental model systems (16). However, immunosuppressive chemotherapeutic agents have also been combined successfully with immunotherapy. It is known that if chemotherapy is administered intermittently there is recovery of immune response between courses of chemotherapy (17). Thus, the principles outlined above for chemoimmunotherapy for melanoma have now been extended to intermittent immunosuppressive chemotherapeutic regimens in acute myelogenous leukemia (18-20), metastatic breast cancer (21), and head and neck cancer.[5] Similar data have been reported by Israel and Edelstein (22) using subcutaneous *C. parvum* with multidrug chemotherapy.

Although it was once speculated that immunotherapy may have no role in patients with far-advanced cancer, the results in this paper demonstrate that this is not so. It is known that these advanced patients have circulating immune complexes which may inhibit effective tumor immunity (23).[6] BCG may work by a variety of mechanisms, including activation of the reticuloendothelial system which can remove these antigen-antibody complexes.

By removal of blocking factors from the serum, effective cell-mediated immunity and/or humoral immunity which is known to exist in malignant melanoma may then become effective (24-26). In addition, BCG has now been demonstrated to cross-react with melanoma antigens accounting, perhaps, for some of its antitumor effects (27). In addition, immunopotentiators have been demonstrated to have the potential to augment colony-forming units in the bone marrow (28); and thus may lead to more rapid recovery from myelosuppression.

The study by Ikonopisov (6) suggests that DTIC chemotherapy can be combined with immunotherapy to prolong the disease-free interval and survival in melanoma patients with minimal residual disease. This confirmed the data by our group and others demonstrating the efficacy of adjuvant immunotherapy alone after surgical removal of metastatic lymph nodes in melanoma (29,30).

One final consideration in the design of immunotherapy and chemotherapy protocols, particularly with DTIC, must be kept in mind. We have recently reported that drug-metabolizing enzyme activities of hepatic microsomes from rats were significantly inhibited after iv administration of BCG or *C. parvum*. Thus, Pasteur BCG injected iv in doses equivalent to that used clinically caused a 20%-45% reduction in DTIC-demethylase activity 10-14 days later. Similarly, the inhibition of the aniline hydroxylase and cytochrome P-450 content of microsomes was reduced. For drugs that are mainly cleared as the unchanged parent compound, such as DTIC, it was likely that the reduced rates of biotransformation would lead to sub-optimal levels of the active metabolites. For drugs that are detoxified by microsomal enzymes, the effect of reduced metabolic activity is more predictable. A prolonged duration of action would probably produce enhanced toxicity. We are currently measuring the effects of immunotherapy on the metabo-

[5]Richman SP, Livingston RB, Suen JY, et al. Chemotherapy versus chemoimmunotherapy of head and neck cancer: report of a randomized study. In press. *Cancer Treatment Reports*, vol 60 (5), 1976.

[6]Rossen R, Reisberg M, Hersh EM, et al. Detection of immune complexes in the sera of cancer patients by the C^{1}Q assay. In preparation.

lism of DTIC patients and we hope to further improve the design of immunotherapy and chemotherapy protocols by such a model study (31).

In conclusion, further research of immunotherapy combined with DTIC chemotherapy seems promising for patients with melanoma and other diseases in which DTIC is an active drug, such as soft tissue sarcoma. The design of innovative programs of chemoimmunotherapy has the potential to yield improved results for the control of human malignancy during the next decade.

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Phase I Evaluation of DTIC (NSC-45388) and Other Studies in Malignant Melanoma in the Central Oncology Group[1,2]

Robert O. Johnson,[3,*] Gerald Metter,[4] William Wilson,[5] George Hill,[6] and Edward Krementz[7,8]

SUMMARY

The major toxic effects of DTIC are nausea and vomiting, leukopenia, and thrombocytopenia. It is an effective drug in the treatment of malignant melanoma with better response rates in women than in men. The addition of other drugs to DTIC therapy does not produce a higher response rate. Preliminary results indicate that as an adjuvant to standard surgical therapy DTIC does not appear beneficial to patients with a high risk for recurrence.

[Cancer Treat Rep 60:183-187, 1976]

In 1967, after completion of initial studies by the National Cancer Institute's animal screening program followed by appropriate pharmacology and pharmaceutic studies, the Central Oncology Group (COG) members initiated a phase I study of DTIC. After re-analysis, this report summarizes the first 200 patients as the phase I group. It briefly reviews the additional 482 patients in our phase II trials and reports on the current status of three malignant melanoma studies—two phase III trials of DTIC compared to three different drug combinations and a surgical adjuvant study in high-risk patients.

Phase I Study of DTIC

Wagner et al (1) have published a summary of the COG phase I-II studies with DTIC. We have reanalyzed the first 200 of these cases as the phase I group using the more stringent COG criteria of 1975. DTIC was given iv in doses which varied from 0.5 to 6.0 mg/kg/day for 10 days. The drug was repeated at monthly intervals if there was a response or no change. Each course of drug was counted for toxicity purposes. The response was counted only once even though different areas of tumor may have responded during different courses of therapy. The evaluation of response was done by the current methods of the COG in which a patient is considered to have had a response if there has been a 50% decrease in the tumor over a period of at least 1 month. Progression was recorded if the tumor doubled in size or if new lesions appeared.

Table 1 is a summary of the toxicity criteria of the COG. These numbers are used in other phase I tables to show the individual patient level of toxicity. Using these criteria the three major toxic effects of DTIC (nausea and vomiting, leukopenia, and thrombocytopenia) are reported in table 2. The

TABLE 1.—COG toxicity criteria

Severity	White blood cell count (cells/mm^3)	Platelet count (cells/mm^3)
0 = None	>4000	>125,000
1 = Mild	3000-4000	100,000-125,000
2 = Moderate	2000-2999	75,000-99,999
3 = Severe	1000-1999	50,000-74,999
4 = Life threatening	<1000	<50,000

[1]Supported by Public Health Service grant CA-12271 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]DTIC: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

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[8]The authors wish to thank Phyllis Multhauf and Scot Moss for their excellent assistance.

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TABLE 2.—DTIC phase I study (major toxic effects)

Dose level (mg/kg)	No. of patients treated	No. of patients with—*		
		Nausea/vomiting†	Leukopenia‡	Thrombocytopenia§
0.5	8	—	—	—
1.0	9	—	—	—
1.5	3	1(2)	1(1)	—
2.0	3	3(0,1,2)	—	—
2.5	7	4(1,1,1,2)	3(1,1,1)	1(2)
3.0	34	4(1,1,2,2)	—	—
3.5	41	2(1,2)	1(1)	1(2)
4.0	52	6(1,1,1,1,1,3)	2(2,3)	1(1)
4.5	74	5(1,1,1,1,2)	2(1,2)	1(3)
5.0	40	8(1,1,1,1,1,1,2,2)	2(1,1)	1(2)
5.5	27	5(1,1,1,2,2)	1(1)	—
6.0	3	1(1)	1(2)	1(2)

*Nos. in parentheses = degrees of toxicity for individual patients. *See* table 1 for definitions.
†Days 1-3.
‡Days of nadir = Days 12-17; days to recovery = 7-14.
§Days of nadir = Days 12-30; days to recovery = 7-14.

individual toxic effects of each patient are recorded according to the toxicity criteria, eg, eight of 40 patients had nausea and vomiting (six were mild [1] and two were moderate [2])when DTIC was given at a dose of 5 mg/kg/day.

Toxic effects appeared at most of the dose levels beginning with 1.5 mg/kg up to 6.0 mg/kg. Similarly, leukopenia occurred in doses ranging from 1.5 to 6.0 mg/kg. Thrombocytopenia was first reported at 2.5 mg/kg. The nausea and vomiting was self-limiting, occurring during the first 3 days with no further evidence of this toxicity until the next course of therapy. Table 3 summarizes other aspects of toxicity. The most common biochemical change was a serum glutamic oxaloacetic transaminase (SGOT) elevation which occurred in approximately half the patients. Patients who had liver disease were not recorded as having change because of the possible confusion between tumor and drug effect. Other toxic effects most commonly noted were a flu-like syndrome, dizziness, chills, and malaise. Many other single, possibly toxic events were recorded in patients but it is difficult to state precisely if the symptoms of toxicity were due to drug or disease.

The response rate during the first course of therapy was 24% and only 8% during the second course (table 4). No responses are recorded thereafter. Table 5 indicates the response rate by sex. There is a statistical difference ($P = 0.01$) with women having the best response rate of 26% compared to men who had only 8%. Our highest response rate occurred in the dose range of 4-5 mg/kg.

The melanoma response rate to DTIC is also analyzed by age (table 5). There is no statistical difference shown in those patients ≤ 54 years of age compared to those > 54 years of age.

TABLE 3.—DTIC phase I study (other toxic effects)

Toxic effect	Occurrence (%)
SGOT elevation	50.5
Abnormal blood urea nitrogen level	17.5
Fever	11.0
Eosinophilia	0.7
Diarrhea	0.5
Other	
Flu-like syndrome	
Dizziness	
Chills	
Malaise	

TABLE 4.—DTIC phase I study (response by course)

Course No.	Total No. of patients	Response rate (%)
1	89	24
2	39	8
3	12	0
4	5	0
5	2	0
Total	147	16

TABLE 5.—DTIC phase I study (response by sex, age, and prior therapy)

Dose level (mg/kg)	Sex				Age				Prior therapy			
	Men		Women		≤ 54 yrs		> 54 yrs		No prior therapy		Prior chemotherapy or radiotherapy	
	No. of patients	Response rate (%)	No. of patients	Response rate (%)	No. of patients	Response rate (%)	No. of patients	Response rate (%)	No. of patients	Response rate (%)	No. of patients	Response rate (%)
2.5	3	0	1	100	1	100	3	0	1	100	3	0
3.0	14	21	8	12	18	17	4	25	3	33	19	16
3.5	14	0	12	17	18	11	8	0	14	14	12	0
4.0	13	0	18	33	22	23	9	11	15	27	16	12
4.5	17	0	16	25	19	16	14	7	19	11	14	14
5.0	12	25	8	38	13	31	7	29	9	44	11	18
5.5	6	0	5	20	6	0	5	20	4	25	7	0
Total	79	8	68	26	97	19	50	12	65	23	82	11

In table 5 the response to DTIC of patients with malignant melanoma is analyzed with regard to prior therapy. Those who had no prior therapy had a 23% overall response rate compared to 11% for those who had prior chemotherapy or radiation therapy. This is significant at the $P = 0.05$ level.

In this same series of 200 patients, there was one response in a lymphoblastic lymphosarcoma, one each in a carcinoma of the pancreas and a carcinoma of the esophagus, one in an unknown primary tumor, and two in colorectal cancer.

DTIC Phase I-II Study

No attempt has been made to re-analyze the additional 482 cases out of a total of 682 patients entered in the original DTIC phase I-II study as reported by Wagner et al (1). In this total study the dose of DTIC used for phase II patients was 4.5 mg/kg/day iv for 10 days. Patients who had received at least 7 days of therapy were considered acceptable for analysis. In the total of 682 patients, 871 courses of therapy were given for 39 different tumor types. The study was conducted over a period of 3 years. Table 6 lists the response as evaluated by Wagner. The criteria for response did not require a response duration of at least 1 month. Therefore, the melanoma response rate is slightly higher than reported in the re-analysis of the first 200 patients of the phase I group. Table 7 shows the response of melanoma by site of metastatic tumor indicating that response can occur in many different organs.

There were 21 patients with melanomas who had a complete response. In this group of responders 18 had skin involvement, 14 had node involvement, and 13 had soft-tissue involvement. There were eight patients having some degree of lung involvement, and only one having some degree of liver involvement. Complete responses were not seen in patients with bony or central nervous system (CNS) disease.

DTIC Versus Combination Therapy (COG-7130)[9]

This randomized trial compares DTIC to three combinations of DTIC with other drugs of possible value in malignant melanoma (2). Table 8 lists the four treatment categories. These treatments were repeated at 6-week intervals. At the present time a total of 267 patients have been entered in the study, of which 240 were evaluable for a 90% evaluability rate. There was a sex difference in treatment

[9]Study Chairmen: Drs. Edward Krementz and George Hill; Statisticians: Dr. Gerald Metter and Scot E. Moss.

TABLE 6.—DTIC phase II study (response by tumor type)

Tumor type	No. of patients	No. of patients responding	Response rate (%)
Melanoma	393	109	28
Colorectal	104	9	9
Epidermoid	34	1	3
Lung	20	3	15
Breast	18	2	11
Osteogenic sarcoma	8	1	—
Anaplastic	8	3	—
Stomach	7	2	—
Bladder	4	2	—
Lymphoma	4	1	—
Liposarcoma	4	1	—

TABLE 7.—DTIC phase II study (response by site)

Site	Total No. of patients	No. of patients with—		Response rate (%)
		No response	Response	
Skin	296	212	84	28
Nodes	243	170	73	30
Soft tissue	211	143	68	32
Lung	159	117	42	26
Liver	72	56	16	22
Bone	33	28	5	15

group 3 which was 71% men. The toxicity was as expected with very few having severe toxic effects. Diarrhea, nausea and vomiting, and leukopenia were more severe in those patients who responded to one of the treatment arms. To date, the response rates recorded in the four treatment arms are: treatment 1 (17%), treatment 2 (18%), treatment 3 (22%), and treatment 4 (11%). A separate analysis has been made of those patients who received immunotherapy prior to chemotherapy. There were 39 such patients; of these four patients responded. The remaining patients were recorded as having no change. The survival of these patients was no different from that of other patients in the study.

Because of the response findings, treatment group 4, which included hydroxyurea,[10] has been discontinued.

Figure 1 indicates the survival by organ involvement. Liver involvement carries the worst prognosis along with brain or CNS involvement. Figure 2 shows that patients who respond to treatment have a longer survival. At 6 months, 62% of the patients who responded are alive compared to 24% of those who did not respond. Similarly, after 1 year 26% of the responders compared to only 4% of the nonresponders are alive.

TABLE 8.—DTIC vs combination therapy (COG-7130)

Treatment No.	Drugs	Dose and schedule (mg/kg/day)
1	DTIC	4.5, × 10
2	DTIC	2.7, iv × 5
	CCNU	1.5, orally Day 2
	Vincristine	0.027, iv Days 1,5
3	DTIC	2.7, iv × 5
	BCNU	2.0, iv Day 2
	Vincristine	0.027, iv Days 1,5
4	DTIC	2.7, iv × 5
	BCNU	2.0, iv Day 2
	Hydroxyurea	30, orally Days 2,5,9,12,16,19

[10]Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1.

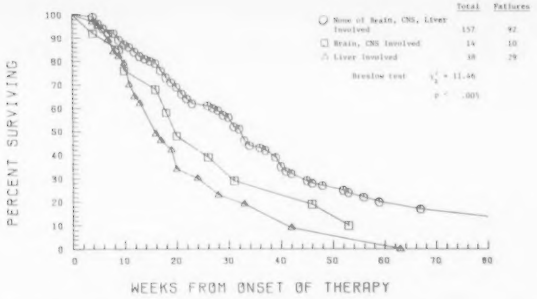

FIGURE 1.—COG-7130: combination therapy for malignant melanoma. Survival by organ involvement.

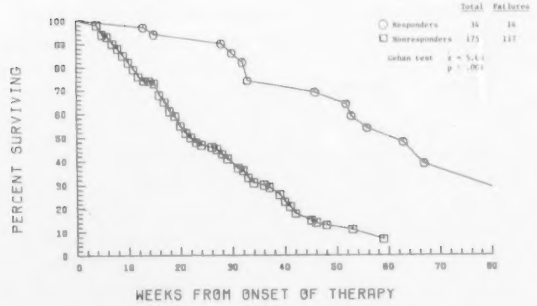

FIGURE 2.—COG-7130: combination therapy for malignant melanoma. Survival by response.

DTIC Versus Combination Therapy (COG-7131)[11]

This study is similar to COG-7130 but changes were made in treatment arms 3 and 4. In treatment 3 the dose of BCNU[12] was increased to 3.0 mg/kg iv on Day 2. In treatment 4 the hydroxyurea was omitted and vincristine[13] was added at a dose of 0.27 mg/kg iv on Days 1 and 5. It is too early at this time to report the results of this study; however, 117 patients have been entered of which 47% have been evaluated. To date, the overall response rate is 17%.

DTIC Melanoma Adjuvant Study (COG-7040)[14]

This is a randomized study of patients who have had a standard surgical procedure for malignant melanoma (3). All patients entered have a relatively poor prognosis. Randomization is to treatment by standard surgery only or surgery plus DTIC (4.5 mg/kg/day iv) for 10 days in months 2, 5, 8, and 11 after surgery.

One of the major considerations in the design of this study was the known severe carcinogenic activity of DTIC in animals as reported by Skibba et al (4). For this reason there was some reluctance to give patients with a good prognosis a large amount of a severe animal carcinogen. To date, we have not seen an unusual number of new cancers in patients treated with DTIC and these patients will be followed for evidence of increased incidence of cancers.

At the present time 133 patients have been entered into this randomized study and 108 have been evaluated. Of this group 107 were considered satisfactory study patients. In treatment group A 54% are free of disease and in treatment group B 47% are free of disease. There presently exists a balance at sex, age, primary site, and institutions within the study. At the present time we are not reporting which mode of therapy is treatment A or B.

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[11]Study Chairmen: Drs. George Hill and Edward Krementz; Statisticians: Dr. Gerald Metter and Scot E. Moss.

[12]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

[13]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

[14]Study Chairman: Dr. George Hill; Statisticians: Dr. John Van Ryzin and Scot E. Moss.

DTIC (NSC-45388) Studies in the Southwest Oncology Group[1,2]

John J. Costanzi[3,*]

SUMMARY

In a phase I study, the best antitumor/toxicity ratio for DTIC was reported to be at a dose of 250 mg/m²/day × 5 repeated at 28-day intervals. Nausea, vomiting, leukopenia, and thrombocytopenia were the major toxic effects noted. The best responses were seen in disseminated melanoma (19%), various sarcomas (22%), and Hodgkin's disease. A subsequent phase II study in refractory lymphomas showed a response rate in Hodgkin's disease of 56%. In disseminated melanomas, DTIC was then combined with vincristine and BCNU and demonstrated a response rate of 23% which did not improve with the addition of chlorpromazine (23%). A response rate of 31% was seen with the combination of DTIC, BCNU, and hydroxyurea which did not improve with the addition of vincristine (30%). Responders had a more significant survival rate as compared to nonresponders.

[Cancer Treat Rep 60:189-192, 1976]

The Southwest Oncology Group (SWOG) performed a phase I study of iv DTIC (1). The drug was administered in 100-250 ml of 5% dextrose and water over a 15-30-minute period for 5 days every 3 weeks. The initial dose level was 70 mg/m²/day and was progressively increased. Myelosuppression and antitumor effect were first noted at 150 mg/m²/day; most patients received a dose of 250 mg/m²/day for 5 days.

The major toxic effect of DTIC was nausea and vomiting which began 1-3 hours after drug administration, was most severe after the first dose, and diminished after subsequent doses on the 5-day course. Leukopenia and thrombocytopenia occurred in equal frequency and were the second most common toxic effects. This myelosuppression was moderately severe at the 350-400 mg/m²/day dose level, but at the 250 mg/m²/day dose level most patients did not have peripheral blood cytopenia and when it did occur, it was relatively mild. When leukopenia occurred it was maximum at 25 days with complete recovery by 40 days. The thrombocytopenia time sequence was similar to leukopenia. Other toxic effects noted were pain along the injected vein (usually occurring after direct iv injection), a "flu-like" syndrome, and a suggestion of hepatic toxicity (reversible enzyme elevations). We therefore recommend that the dose level for DTIC used alone should be 250 mg/m² for 5 days.

In this phase I study 171 patients were evaluable for response (table 1). A complete response was defined as the disappearance of all evidence of tumor and a partial response was defined as a > 50% reduction in measurable tumor. The tumor types and response rates are noted in table 2. There was a response rate of 19% (21 of 110) for melanomas and 22% (five of 23) for various sarcomas. A response was noted in one of two patients with Hodgkin's disease.

The efficacy of DTIC in the treatment of lymphoma was further pursued by the SWOG. A phase II study in patients with advanced refractory lymphoma was performed (2). The initial dose level was 250 mg/m²/day for 5 days, repeated at 3-week intervals. Subsequent doses were increased to 300-350 mg/m²/day (if no myelosuppression occurred) and were not changed or reduced depending on the level of myelosuppression. The response rate of the various lymphomas is noted in table 3. Of 18 patients with Hodgkin's disease one complete and nine partial responses were noted (56%). The complete response duration was 30+ months, with

[1]Supported in part by Public Health Service grant CA-03096 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

All drugs used in these studies were supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

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TABLE 1.—Patients evaluable for responses to DTIC*

No. of patients—	
Treated	202
Not evaluable	31
Received dose < 150 mg/m²/day × 5	5
Early death from disease (< 28 days)	18
No measurable disease	2
Lost to followup	4
Prolonged delay between courses	2
Evaluable	171

*From ref 1.

three others having a response duration of 10+ months. Although four of 15 patients with lymphosarcoma and one of one patient with mycosis fungoides responded, these responses were brief. No response was noted in 13 patients with reticulum cell sarcoma. The efficacy of DTIC noted in Hodgkin's disease probably represents minimum figures since those patients had extensive prior therapy.

TABLE 2.—Tumor types and responses*

Tumor type†	No. of responses/ No. of patients treated (%)
Melanoma	21/110 (19)
Sarcomas	
Leiomyosarcoma	2/10
Fibrosarcoma	1/7
Rhabdomyosarcoma	1/2
Osteogenic sarcoma	1/2
Ewing's sarcoma	0/1
Undifferentiated sarcoma	0/1
Total	5/23 (22)
Lung	
Squamous cell	1/3
Oat cell	1/1
Adenocarcinoma	0/3
Alveolar cell	0/1
Hodgkin's disease	1/2
Colon	0/4
Breast	0/4
Hypernephroma	0/2
Wilms' tumor	0/2
Embryonal carcinoma (testis)	0/2

*From ref 1.

†One each with the following tumor types had no response: cytosarcoma phylloides, mesothelioma, hepatoma, adenocarcinoma of stomach, transitional cell carcinoma of bladder, unclassified carcinoma, choriocarcinoma of testis, adenocarcinoma of pancreas, adenocarcinoma of maxillary sinus, lymphosarcoma, and squamous cell carcinoma of lip.

Further experience with DTIC in sarcomas is discussed in another paper in this issue. We will discuss the SWOG experience using DTIC in combination with other compounds for the treatment of disseminated malignant melanoma.

BCNU has reported activity in malignant melanoma (3) which appears to increase when combined with the nonmyelosuppressive compound, vincristine.[4] The SWOG has studied the combination of BCNU, vincristine, and DTIC at the following dose levels: BCNU, 150 mg/m² on Day 1; vincristine, 1.4 mg/m² (2 mg maximum) on Days 1 and 5; and DTIC, 150 mg/m²/day iv for 5 days (4). Repeat courses were administered at 28-day intervals. One hundred and thirty-five patients were evaluable for this study. Complete responses were noted in nine of 135 (6.6%) and partial responses were noted in 22 of 135 (16.3%) for a total response rate of 23%. The best responses were noted in patients whose metastases were in the extremities (39%), trunk (26%), gastrointestinal tract (25%), lung (24%), and head and neck (24%). Patients with bone and liver metastases had response rates of 18% and 14% respectively. No responses were noted in patients with brain metastases.

This study compared the final response with the response noted at the completion of the first course (table 4). In patients with documented improvement (tumor decrease of at least 20%) or probable improvement (documented improvement without tumor measurements) after one course, a good final response is to be anticipated. No change or increasing disease after the first course heralded a poor final response.

Chlorpromazine, a phenothiazine, has a marked affinity for melanin and was reported to inhibit the growth of B16 and Harding-Passey mouse melanomas (5). Therefore, the SWOG studied the addition of chlorpromazine to the previously described three-drug regimen of BCNU, vincristine, and DTIC (6). Chlorpromazine was administered orally in a dose of 50 mg three times daily for the 5-day course. Of 142 evaluable patients, 12 (10.4%) achieved a complete response and 15 (13%) a partial response for a total response rate of 23.4%. The addition of chlorpromazine did not increase the efficacy of the three-drug regimen. In this study, some interesting prognostic correlations were noted. Patients whose absolute lymphocyte count was >3000 cells/mm³ had a 50% response rate

[4]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

TABLE 3.—Response of patients with lymphoma to DTIC*

Disease	No. of patients	No. of remissions			Duration of remission (mos)
		Complete	Partial	Total (%)	
Hodgkin's disease	18	1	9	10(56)	2,2+,2+,3,3,4, 4+,10+,12+,30+
Reticulum cell sarcoma	13	0	0	0(0)	—
Lymphosarcoma	15	1	3	4(27)	2,2,2+,3
Mycosis fungoides	1	0	1	1	2
Totals	47	2	13	15(32)	

*From ref 2.

and those with < 3000 cells/mm^3 had a 10%-25% response rate. Those patients with absolute eosinophil counts > 300 cells/mm^3 had a response rate of 35%, while those with < 300 cells/mm^3 had a 20% response rate. As noted for the previous combination chemotherapy study (BCNU, vincristine, and DTIC), a correlation existed between the response after the first course of chemotherapy and the final response.

Cumulative experience with hydroxyurea[5] used as a single agent in 147 patients with melanoma reported a response rate of 21% (7). The SWOG completed a study (8) comparing BCNU, hydroxyurea, and DTIC with BCNU, hydroxyurea, DTIC, and vincristine. In an updating of this published study, 210 evaluable patients received BCNU, hydroxyurea, and DTIC, with 20 (9%) achieving a complete response and 46 (22%) achieving a partial response for a total response rate of 31%. Of the 215 evaluable patients receiving BCNU, hydroxyurea, DTIC, and vincristine, 7% had a complete response and 23% a partial response with a total response rate of 30%. Therefore, vincristine does not add to the efficacy of this combination.

The majority of responders had melanoma metastatic to the skin, subcutaneous tissue, lung, and lymph nodes. The poorest responses were noted in patients with liver and brain metastases. Figure 1 compares the survival curves from the first day of treatment to the last followup for the two regimens—BCNU, hydroxyurea, and DTIC versus BCNU, hydroxyurea, DTIC, and vincristine. The median survival time is 7.0 months for the three-drug regimen and 6.0 months for the four-drug regimen (P = 0.86, not significant). If survival was assessed to be exponential in the two groups, the death rate per month would be approximately 9%. Figure 2 compares the survival curves from the date of response to the date of last followup for the responders. The median survival time is 9 months for partial responders and 20 months for complete responders ($P < 0.01$). In general, female patients have a longer survival time than males (P = 0.08). The major toxic effects mirrored those of the other combinations—nausea and vomiting (45%), leukopenia (34%), and thrombocytopenia

[5]Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1.

TABLE 4.—Response to first course of BCNU, vincristine, and DTIC combination chemotherapy vs final response

Disease status	No. of patients	Final response (%)			
		Complete	Partial	None	Increasing disease
Documented improvement	21	33	57	5	5
Probable improvement	29	28	48	10	14
No change	12	0	8	33	58
Increasing disease	59	0	3	8	88

*From ref 4.

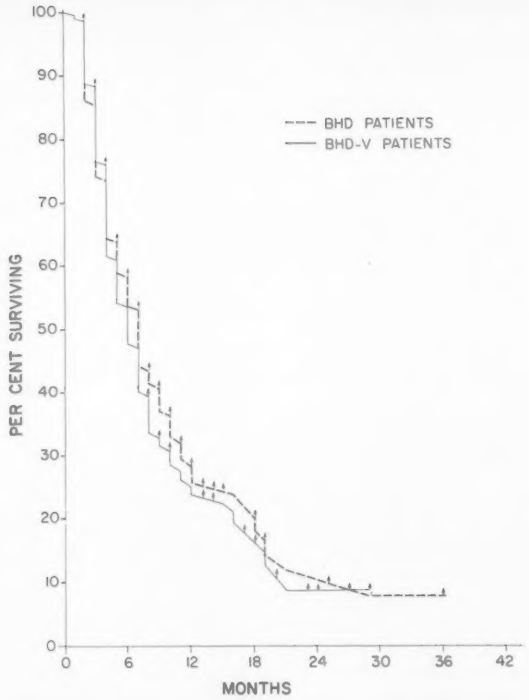

FIGURE 1.—Comparative survival curves from 1st treatment day to the last followup: BCNU, hydroxyurea, and DTIC (BHD) vs BCNU, hydroxyurea, DTIC, and vincristine (BHD-V).

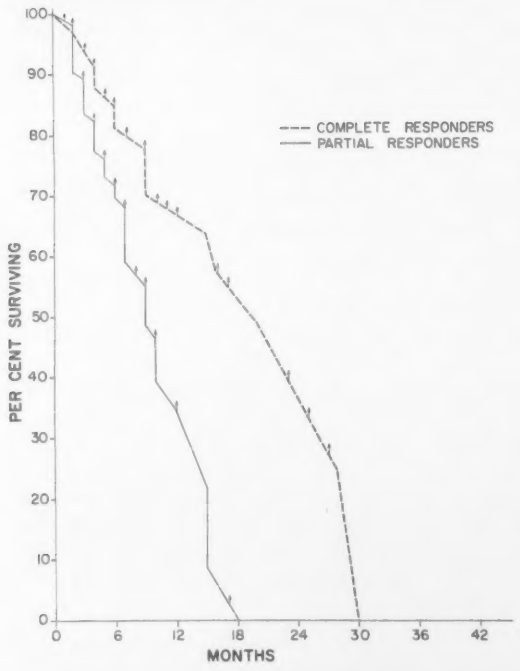

FIGURE 2.—Comparative survival curves from date of response to last followup: complete vs partial responders.

(20%). In the four-drug combination regimen group, peripheral neuropathy was noted in 10%.

The SWOG studies using DTIC in melanoma are listed in table 5.

TABLE 5.—SWOG experience with DTIC alone and in combination for melanoma

Drugs	Response rate (%)
DTIC	19
DTIC + vincristine + BCNU	23
DTIC + vincristine + BCNU + chlorpromazine	23
DTIC + vincristine +BCNU + hydroxyurea	30
DTIC + BCNU + hydroxyurea	31

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Eastern Cooperative Oncology Group Studies With DTIC (NSC-45388)[1]

Paul P. Carbone[2,*] and William Costello[3]

SUMMARY

In four randomized controlled clinical trials with DTIC involving patients with malignant melanoma, the Eastern Cooperative Oncology Group has seen a consistent objective response rate of 16%. A study of combinations of DTIC with two nitrosoureas indicated no additive effect. Survival and response were strongly correlated with sex, ambulatory status, and site of disease involvement. As secondary treatment DTIC was less effective, indicating only a 3% response rate. Survival benefit could be related to response. Among the ten largest contributors of patients, response rates varied from 3% to 40%. Similarly, while the overall response rate was not different for the combinations as compared to DTIC alone, the variation noted from study to study reflects the need for controlled randomized trials of chemotherapy for patients with malignant melanoma.

[Cancer Treat Rep 60:193-198, 1976]

Since 1968, the Eastern Cooperative Oncology Group (ECOG) has undertaken four controlled randomized studies with DTIC, all involving patients with malignant melanoma (1-4). This report summarizes the results of these trials for DTIC alone or in combination. During this period, we have seen a consistent response rate for DTIC alone, a relative lack of additive effect when DTIC was combined with BCNU or methyl-CCNU,[4] a uniformity of patient survival for all studies, and a relationship between response and site of disease, sex, and ambulatory status. The studies also show that DTIC is less effective as crossover treatment and that survival correlates highly with the degree of responsiveness.

In the first study (EST 0868) an attempt was made to investigate the effectiveness of two dosage schedules with regard to response and survival (1). The observations indicated that with high-dose DTIC (200 mg/m^2 daily × 10) toxicity was greater but total response, completeness of response, and survival were not improved compared to low-dose DTIC (80 mg/m^2 daily × 10).

Subsequent studies involving DTIC are shown in table 1 (2,4). A crossover was built in for patients not receiving DTIC as the first treatment. All patients had measurable metastatic melanoma, an estimated survival of > 2 months, initial white blood cell counts ≥ 5000 cells/mm^3, initial platelet counts ≥ 150,000 cells/mm^3, no prior therapy within the previous 4 weeks before entering the study, and a normal blood urea nitrogen level. In studies EST 1071 and EST 1672, patients were stratified to treatment regimens on the basis of prior or no prior chemotherapy.

The criteria for response have been previously published (1,2,4). The overall objective response rates of the induction arm and the crossover arm are shown in table 2. The denominator used in calculating these responses included all evaluated patients; no patient was excluded because of early death or inadequate treatment. The combined response rate for DTIC alone in the three studies was 16% for the primary induction therapy and 3% for the crossover therapy ($P < 0.001$). No differences in the response rates were seen for patients when BCNU or methyl-CCNU was combined with DTIC.

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[1]DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

[2]Eastern Cooperative Oncology Group (chairman), Silver Spring, Md.

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[4]Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

BCNU: NSC-409962; CAS reg. No. 154-98-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

TABLE 1.—ECOG DTIC studies

Study No.	Drugs	Schedule
EST 0170	DTIC	150 mg/m² daily × 5 q25d
	DTIC	100 mg/m² daily × 5
	+	
	BCNU	75 mg/m² Days 2 and 3
EST 1071	DTIC	150 mg/m² daily × 5 q30d
	TIC*	800 mg/m² daily × 5 q30d
EST 1672	DTIC	200 mg/m² daily × 5 q21d
	DTIC	150 mg/m² daily × 5 q21d
	+	
	Methyl-CCNU	130 mg/m² Day 2 q6wks
	Methyl-CCNU	200 mg/m² Day 1 q6wks

*TIC: NSC-60339; CAS reg. No. 70-09-7; terephthalanilide, 2-chloro-4', 4''-di-2-imidazolin-2-yl-.

In all studies response was a function of various factors, ie, sites of involvement (P = 0.029), sex (P = 0.022), and ambulatory status (P = 0.016). However, response was not related to the age of the patients, prior treatment, or time from first symptoms (table 3).

Since ten institutions were large contributors to all the studies, we were able to examine the variability of response from study to study by institution. Table 4 shows the response rates to DTIC alone for the ten largest contributing institutions. The response rates varied from 3% to 40% with an overall rate of response of 16%. The overall response rates for DTIC, DTIC plus BCNU, and DTIC plus methyl-CCNU were not different from each other. Thus, if the control arm was not used in studies EST 1071 and EST 1062, three different conclusions could be drawn indicating the superiority of each of the regimens by picking the appropriate institution. We believe that these data are important when considering reports of unrandomized sequential studies with DTIC in combination with other nitrosoureas.

TABLE 2.—Overall objective tumor response rates in ECOG melanoma studies

	CR + PR/total No. of patients (No. of CRs)	
Drugs	Induction	Crossover
DTIC alone	45/275,16% (13)	3/92,3% (1)
DTIC + BCNU	16/93,17% (7)	—
DTIC + methyl-CCNU	18/114,16% (8)	0/3
Total	79/482,16% (28)	3/95,3% (1)

Not only were the responses quite similar but the survival experience was consistent, with a median survival of 19.6 weeks for the induction phase (table 5). The median survival was about 4 weeks shorter for crossover with DTIC, reflecting the median duration on study for the initial treatment. Survival was also highly dependent on extent of disease, ambulatory status, and quality of objective response ($P < 0.001$) (table 6).

The duration of response for the three studies is indicated in table 7. The median duration of response for DTIC varied from study to study, ranging from 11.6 to 20.7 weeks with an overall median of 15.8 weeks. Although the combination of DTIC plus methyl-CCNU appears to be associated with longer durations, there were not enough responders to ascertain true differences (P = 0.53 against DTIC alone and P = 0.29 against DTIC plus BCNU). Figure 1 illustrates the remission duration data for the three regimens.

The effect on survival of quality of response, ambulatory status, disease sites, and time until progression is illustrated in figures 2-6. The most impressive effect on survival of complete responders and partial responders for DTIC is shown in figure 5, which indicates that although response rates may be low (16%), the response is important to the patient.

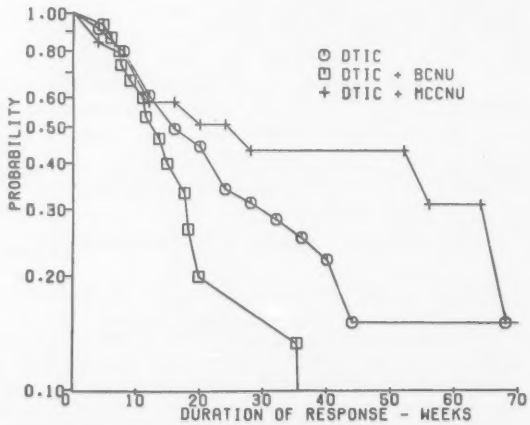

FIGURE 1.—Duration of response for patients receiving induction therapy with DTIC, DTIC + BCNU, and DTIC + methyl-CCNU. The increased duration from DTIC + methyl-CCNU is not statistically significant.

TABLE 3.—Overall objective tumor response rates in ECOG melanoma studies (EST 0170, 1071, and 1672) using DTIC as an induction agent

Prognostic factors	No. of CR + PR/total No. of patients			
	DTIC alone	DTIC + BCNU	DTIC + methyl-CCNU	Total
Extent of disease				
Soft tissue only	17/69,25%	4/26,15%	6/25,24%	27/120,23%
Extensive	26/198,13%	12/67,18%	8/78,10%	46/343,13%
Study				
EST 0170	8/66,12%	16/93,17%	—	24/159,15%
EST 1071	17/84,20%	—	—	17/84,20%
EST 1672	20/125,16%	—	18/114,16%	38/239,16%
Age				
< 40	10/56,18%	2/25,8%	5/23,22%	17/104,16%
40-49	10/52,19%	6/16,38%	2/27,7%	18/95,19%
50-55	9/50,18%	3/13,23%	4/15,27%	16/78,21%
56-65	7/58,12%	3/12,25%	3/30,10%	13/100,13%
> 65	7/52,13%	2/22,9%	3/19,16%	12/93,13%
Sex				
Male	22/156,14%	6/53,11%	6/55,11%	34/264,13%
Female	21/112,19%	10/35,28%	11/53,21%	42/200,21%
Prior surgery				
No	7/36,19%	2/8,25%	1/13,8%	10/57,18%
Yes	36/232,16%	14/80,17%	16/95,17%	66/415,16%
Prior radiation therapy				
No	36/227,16%	15/80,19%	16/97,16%	67/404,17%
Yes	7/40,18%	1/8,13%	1/11,9%	9/59,15%
Prior chemotherapy				
No	37/230,16%	14/73,19%	14/86,16%	65/389,17%
Yes	6/37,16%	2/15,13%	3/22,14%	11/74,15%
Initial ambulatory status				
Ambulatory	31/169,18%	10/45,22%	12/75,16%	53/289,18%
Nonambulatory	6/73,8%	6/47,13%	3/31,10%	15/151,10%
Time since 1st symptoms				
< 1 yr	6/61,10%	4/23,17%	4/18,22%	14/102,14%
1-2 yrs	13/56,23%	5/16,31%	5/27,19%	23/99,23%
2-4 yrs	11/50,22%	0/17,0%	3/29,10%	14/96,15%
> 4 yrs	7/43,16%	1/8,13%	3/18,17%	11/69,16%
Total	45/275,16%	16/93,17%	18/114,16%	79/482,16%

TABLE 4.—Overall objective tumor response rates in ECOG melanoma studies (EST 0170, 1071, and 1672) using DTIC as an induction agent

Institute	CR + PR/total No. of patients			
	DTIC alone	DTIC + BCNU	DTIC + methyl-CCNU	Total
1	4/10, 40%	0/6,0%	1/3,33%	5/19,26%
2	1/14,7%	2/11,18%	2/4,50%	5/29,17%
3	1/30,3%	3/17,18%	0/11,0%	4/58,7%
4	4/14,29%	1/6,17%	1/7,14%	6/27,22%
5	2/19,11%	0/3,0%	1/6,17%	3/28,11%
6	2/8,25%	1/3,0%	0/4,0%	3/15,20%
7	9/28,32%	0/12,0%	1/7,14%	10/47,21%
8	9/29,31%	5/8,63%	0/10,0%	14/47,30%
9	2/17,12%	0/2,0%	3/9,33%	5/28,18%
10	2/19,11%	2/13,15%	3/5,60%	7/37,19%
All others (26 institutions)	9/87,10%	2/12,17%	6/48,13%	17/147,12%
Total	45/275,16%	16/93,17%	18/114,16%	79/482,16%

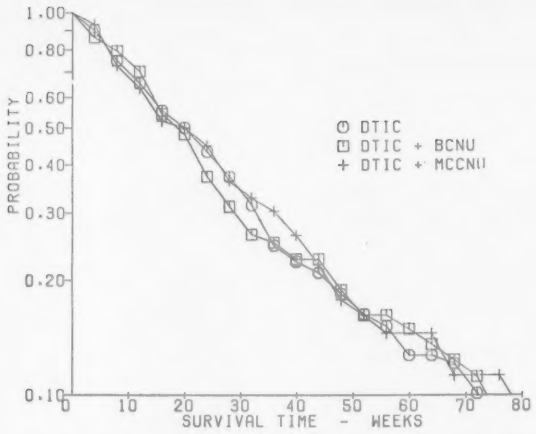

FIGURE 2.—Overall survival of patients treated with DTIC, DTIC + BCNU, and DTIC + methyl-CCNU.

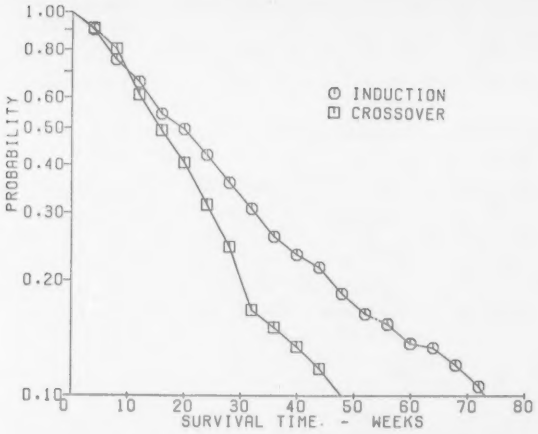

FIGURE 3.—Survival of patients receiving DTIC as primary induction or crossover therapy ($P = 0.014$).

TABLE 5.—Survival data

Drugs	No. of patients/median survival time in wks	
	Induction	Crossover
DTIC alone	275/19.9	92/16.0
DTIC + BCNU	92/18.7	—
DTIC + methyl-CCNU	114/19.9	3/10.3

DISCUSSION

The ECOG has been able to mount four separate studies utilizing DTIC for patients with melanoma. These studies have indicated that a consistent response rate of 16% appears realistic by evaluating a total of 275 patients treated with this agent alone. The 95% confidence interval for the response rate is 12%-20.8%. This response rate is not much different than that reported by Carter

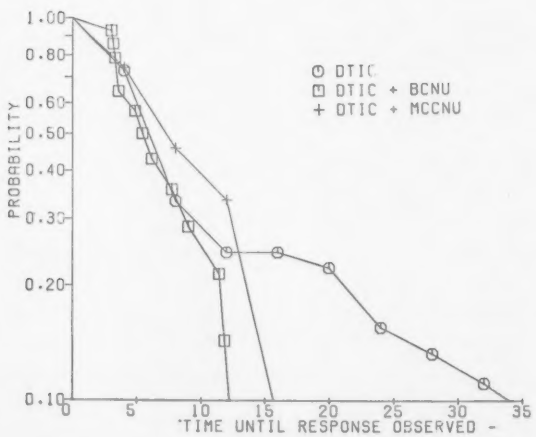

FIGURE 4.—Time until response to DTIC, DTIC + BCNU, and DTIC + methyl-CCNU.

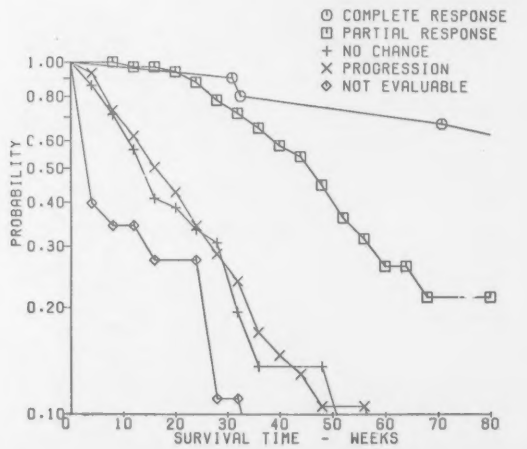

FIGURE 5.—Survival of patients receiving DTIC as primary induction therapy as a function of quality of response.

TABLE 6.—Survival in ECOG melanoma studies (EST 0170, 1071, and 1672) using DTIC as an induction agent

Prognostic factors	No. of deaths/total No. of patients, median survival time		
	DTIC alone	DTIC + BCNU	DTIC + methyl-CCNU
Extent of disease			
Soft tissue only	51/69,33.0 wks	21/25,19.2 wks	18/25, 26.6 wks
Extensive	175/197,15.3 wks	60/67,18.2 wks	66/78,14.5 wks
Initial ambulatory status			
Ambulatory	137/168,26.1 wks	40/45,22.8 wks	54/75,26.2 wks
Nonambulatory	69/73,9.4 wks	40/46,13.3 wks	29/31,6.7 wks
Objective response			
Complete	4/13,182.5 wks	5/7,36.5 wks	3/8,44.9 wks
Partial	23/32,45.6 wks	9/9,29.6 wks	6/10,37.6 wks
No change	39/41,13.5 wks	17/20,16.2 wks	11/12,6.7 wks
Progression	150/172,16.2 wks	41/46,15.8 wks	62/76,15.6 wks
Response not evaluable	14/16,3.0 wks	9/10,2.7 wks	6/8,3.1 wks
Total (including those with unknown factors)	231/275,19.9 wks	81/92,18.7 wks	88/114,19.9 wks

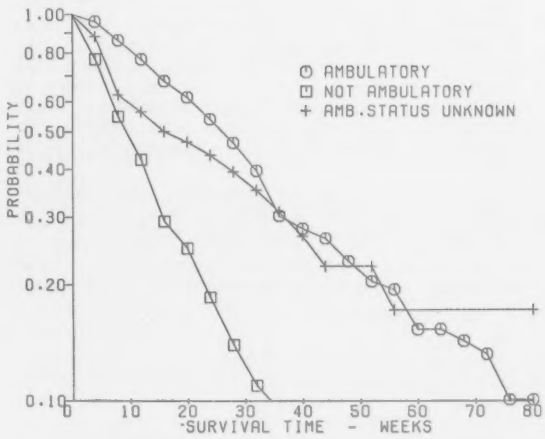

FIGURE 6.—Survival of patients receiving DTIC as primary induction therapy as a function of ambulatory status ($P < 0.001$).

and Friedman in a recent review of DTIC (5). Thus DTIC is a major therapeutic agent in the treatment of patients with metastatic melanoma.

Our results also indicate that response and survival are strongly correlated with certain clinical features of the patient. These factors include sex, soft tissue disease, and ambulatory status. Thus, variability in response may occur because of intrinsic features of the patients rather than as a primary result of drug treatment. We have also shown that response to DTIC may vary considerably from one hospital to another. Moreover, since the addition of BCNU and methyl-CCNU to DTIC did not improve the overall response rate, we compared the results of the various regimens at a single institution across several studies. Any one of several conclusions could be drawn indicating superiority for each of the regimens.

As with other tumors where response rates are low, evaluation of median survivals does not ade-

TABLE 7.—Duration of response in ECOG melanoma studies

Drugs	No. of relapses/No. of CR + PR, median duration of CR + PR			
	EST 0170	EST 1071	EST 1672	Total
DTIC alone	8/8,11.6 wks	13/17,20.7 wks	14/29,16.6 wks	35/54,15.8 wks
DTIC + BCNU	14/15,12.5 wks	—	—	14/15,12.5 wks
DTIC + methyl-CCNU	—	—	11/18,24.5 wks	11/18,24.5 wks
Total	22/23,13.0 wks	13/17,20.7 wks	25/47,18.6 wks	60/87,15.7 wks

quately reflect the activity of an agent. Our data clearly confirm the value of DTIC by examining the survival of responders versus nonresponders. DTIC has a marked effect on improving survival for the 16% of patients who do respond.

In our studies the failure to achieve a higher response rate for the combinations of DTIC plus BCNU and DTIC plus methyl-CCNU is disappointing. Since both BCNU and methyl-CCNU are effective agents in patients with melanoma (3,5-7), the lack of response to DTIC as a crossover drug for previous treatment failures is suggestive evidence that these agents may have similar mechanisms of action. Confirmatory data of cross resistance by DTIC-resistant tumors for BCNU have not been reported (8). CCNU[5] and DTIC have been shown not to be synergistic by Schabel (9). Another cause for the lack of a synergistic effect of DTIC and methyl-CCNU or BCNU might be improper scheduling or dosages of the two-drug combinations. Although not statistically significant, the improved duration of response and the number of complete responses for DTIC plus methyl-CCNU indicate that there may be some advantage to this combined program and further study is indicated. The challenge is to devise effective combinations that may improve the response rates so that more patients benefit. A different approach would be to use DTIC alone or DTIC plus methyl-CCNU as an adjuvant program for those patients who have a high risk of recurrence following initial resection.

[5]CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

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Role of DTIC (NSC-45388) in the Chemotherapy of Sarcomas[1,2]

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SUMMARY

DTIC was one of the first of several new agents evaluated by the Southwest Oncology Group (SWOG) and the M.D. Anderson Hospital and Tumor Institute (MDAH) in the therapy of adult patients with metastatic sarcomas. It yielded an overall response rate of 17%. This is similar to that seen in a review of 138 patients who represent the total number reported in either published or unpublished data. The subsequent addition of DTIC to adriamycin in several studies carried out at the MDAH and in the SWOG has increased the complete and partial remission rate over that seen with adriamycin alone, and more importantly has increased remission duration and survival. DTIC is currently used in combination with adriamycin in the first-line therapy of patients with metastatic sarcomas and should be considered for patients who have relapsed on primary therapy not including DTIC.

[Cancer Treat Rep 60:199-203, 1976]

DTIC has been used in the therapy of adult patients with disseminated sarcomas at the M.D. Anderson Hospital and Tumor Institute (MDAH) and in the Southwest Oncology Group (SWOG) since its initial phase II trial which began in 1969 (1). The results of these sequential studies have been recently reviewed (2). Although the backbone of our current chemotherapy of disseminated sarcomas is clearly adriamycin,[4] the addition of DTIC to our adriamycin-containing regimens has resulted in a modest increase in response rate and a substantial increase in remission duration and survival. It is the purpose of this paper to: *(a)* review the data on the use of DTIC as a single agent in the treatment of sarcomas; and *(b)* present previously unpublished survival data on sarcoma patients treated in the SWOG with adriamycin with and without DTIC.

LITERATURE REVIEW

Data on the use of DTIC as a single agent in the treatment of patients with sarcomas were obtained from the published literature and from unpublished data on file with the Division of Cancer Treatment, National Cancer Institute (NCI) (table 1) (1,3-10).[5-8] Kaposi's sarcoma is by far the most responsive of

[1]Supported in part by Public Health Service grants CA-10379, CA-14028, CA-07177, CA-08096, CA-04915, CA-12644, CA-13238, CA-13236, CA-12213, CA-04920, CA-15467, CA-12014, and CA-03754 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]DTIC: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,-3-dimethyl-1-triazeno)-.

[3]This study was performed by the Southwest Oncology Group (SWOG), *Barth Hoogstraten,* chairman. The following institutions participated in this study: The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute at Houston (*Drs. Gottlieb* [deceased], *Sinkovics, Blumenschein, Benjamin, Bodey, Rodriguez, Burgess,* and *Moon* [assistant biometrician, SWOG]); Wayne State University Medical School, Detroit, Mich (*Dr. Baker*); Henry Ford Hospital, Detroit, Mich (*Dr. O'Bryan*); University of Kansas Medical Center, Kansas City (*Dr. Hoogstraten*); Southern Nevada Memorial Hospital, Las Vegas (*Dr. Quagliana*); The Tumor Institute of the Swedish Hospital Medical Center, Seattle, Wash (*Dr. Rivkin*); University of New Mexico School of Medicine, Albuquerque (*Dr. Saiki*); Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, San Antonio, Tex (*Dr. Coltman*); The Diagnostic Clinic of Houston, Tex (*Dr. Sullivan*); University of Mississippi Medical Center, Jackson (*Dr. Thigpen*); Oklahoma Medical Research Foundation, Oklahoma City (*Dr. Bottomley*); *Ohio State University Hospitals, Columbus (Dr. Balcerzak).*

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[4]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

[5]Western Cancer Study Group minutes, Jan 1971.

[6]SWOG minutes, Oct 1, 1970.

[7]Gottlieb JA. Unpublished data, MDAH.

[8]Data on file. Division of Cancer Treatment, NCI. Source not specified.

TABLE 1.—DTIC in sarcomas: literature survey

Type	No. of patients— Treated	With CR	With PR	Total remission rate (%)	Ref. No.
		Published data			
Leiomyosarcoma	24	1	5	25	1,5 (footnotes 6 and 7)
Fibrosarcoma	18	0	1	6	1,9 (footnote 6)
Osteogenic sarcoma	14	0	2	14	1,4,9,10 (footnote 6)
Rhabdomyosarcoma	13	0	2	15	1,7 (footnotes 6 and 7)
Kaposi's sarcoma	10	3	2	50	8
Liposarcoma	7	0	1	14	9 (footnotes 5 and 6)
Unspecified	6	0	0	—	1,3,4,6,10 (footnote 6)
Ewing's sarcoma	3	0	0	—	1,3,10 (footnote 7)
Mesothelioma	1	0	1	—	4
Chondrosarcoma	1	0	0	—	Footnote 6
Synovial cell sarcoma	1	0	0	—	Footnote 6
Angiosarcoma	1	0	0	—	4
		Unpublished (NCI cumulative) data			
Unspecified	39	0	7	18	Footnote 8
Total	138	4	21	18	

the sarcomas to treatment with DTIC with three CRs and two PRs reported out of ten patients treated at the Uganda Cancer Institute, for an overall remission rate of 50% (8). Leiomyosarcoma is the only one of the more common sarcomas with a response rate to DTIC > 15%. One CR and five PRs have been reported out of 24 patients treated, for a remission rate of 25% (1,5).[6,7] Responses in the order of 15% or less have also been reported in patients with fibrosarcoma, osteogenic sarcoma, rhabdomyosarcoma, liposarcoma, and undifferentiated or unspecified sarcomas (table 1).

STUDIES AT THE MDAH AND THE SWOG

Systematic studies of the treatment of adult patients with disseminated sarcomas were instituted at the MDAH and in the SWOG in 1969. DTIC was one of the first agents studied. One CR and eight PRs were noted among 53 patients treated (1,5).[6,7] No patient with Kaposi's sarcoma was included in the study. The best responses were seen in the 23 patients with leiomyosarcoma (one achieved CR and four achieved PR for a response rate of 23%). A response rate of 31% was obtained in 49 patients with sarcomas treated with adriamycin in the initial phase II evaluation by the SWOG (SWOG-442) (11). A similar response rate was seen in an equivalent number of patients treated 3 years later in the SWOG dose-response study of adriamycin (SWOG-7206) (2). Survival of the two groups was also similar. In 1971 the first study (SWOG-445) combining adriamycin and DTIC was initially undertaken at the MDAH and later in the SWOG (12). Two hundred fifty-four patients were entered in the study and 218 were finally evaluable. The remaining 36 could not be evaluated largely because of major protocol violations or failure to complete one course of chemotherapy due to death from reasons other than tumor or drug toxicity. Twenty-five patients had CR and 67 had PR for an overall response rate of 42%. An additional 33% had no change and 25% had continued progressive disease (2). In the next study (SWOG-7210) vincristine[9] was added to the regimen of adriamycin and DTIC (13,14). No change in response rate or survival was noted in the 107 patients who could be evaluated from the 124 registered patients in the protocol. Since there were no differences between the survival of patients treated with adriamycin alone in SWOG-442 and SWOG-7206, and since there were no differences in survival between the patients treated with adriamycin and DTIC with or without vincristine in SWOG-445 and SWOG-7210, we compared the survival of the 97 patients treated with adriamycin alone against that of the 325 patients treated with adriamycin in combination with DTIC (fig 1). The differences in survival are statistically significant ($P = 0.001$), showing superior survival for the patients treated

[9]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

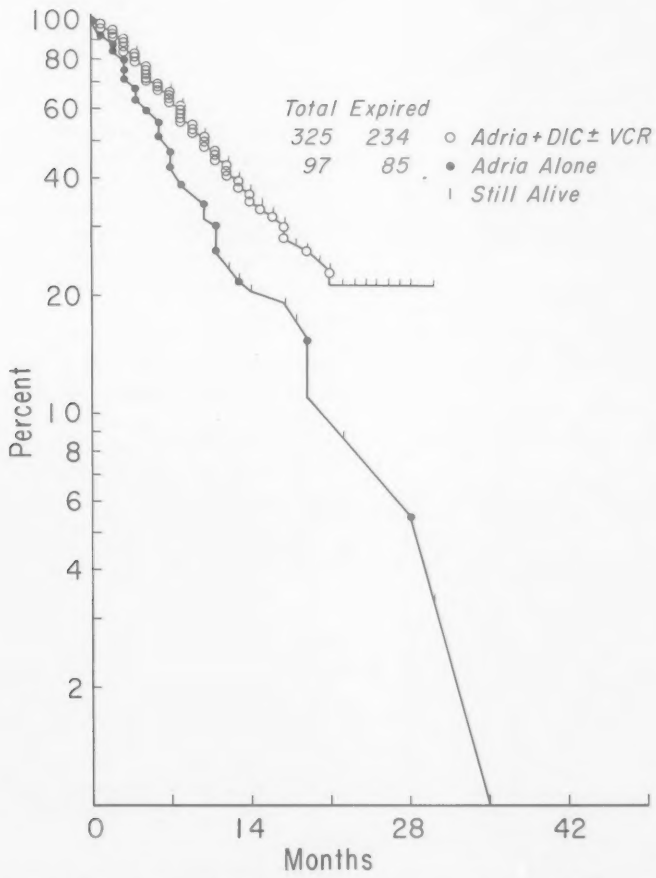

FIGURE 1.—Survival of evaluable sarcoma patients treated with adriamycin alone (SWOG-442 and SWOG-7206) or adriamycin combined with DTIC with or without vincristine (SWOG-445 and SWOG-7210).

with adriamycin in combination with DTIC (median, 10 mos) compared to the patients treated with adriamycin alone (median, 6 mos). Approximately 12% of all patients entered (equally distributed in all four studies) are omitted from these curves. They represent major protocol violations or very early deaths from nondrug or nontumor causes. If these patients were included, the medians would decrease by < 1 month and would not alter the statistical conclusions. Figure 2 considers only the responders in the two groups of protocols. Once again, patients receiving adriamycin in combination with DTIC had statistically significant improvement in survival ($P = 0.01$) with a median of 15 months for the combination regimens versus a median of 8 months for the single-agent therapy. These data convincingly show that the addition of DTIC to adriamycin in the therapy of patients with disseminated sarcomas results in superior survival. Subsequent studies at the MDAH and in the SWOG have been designed to determine whether cyclophosphamide will further increase the response rate and survival of such patients. It is too early to state whether this will or will not be the case.

A small series of sarcoma patients was treated at the Baltimore Cancer Research Center (BCRC), NCI, with adriamycin alone with a remission rate of 41% (15). Therefore, for a time the role of DTIC in the therapy of such patients was viewed with considerable scepticism. After several years of followup, however, recent analysis of remission dura-

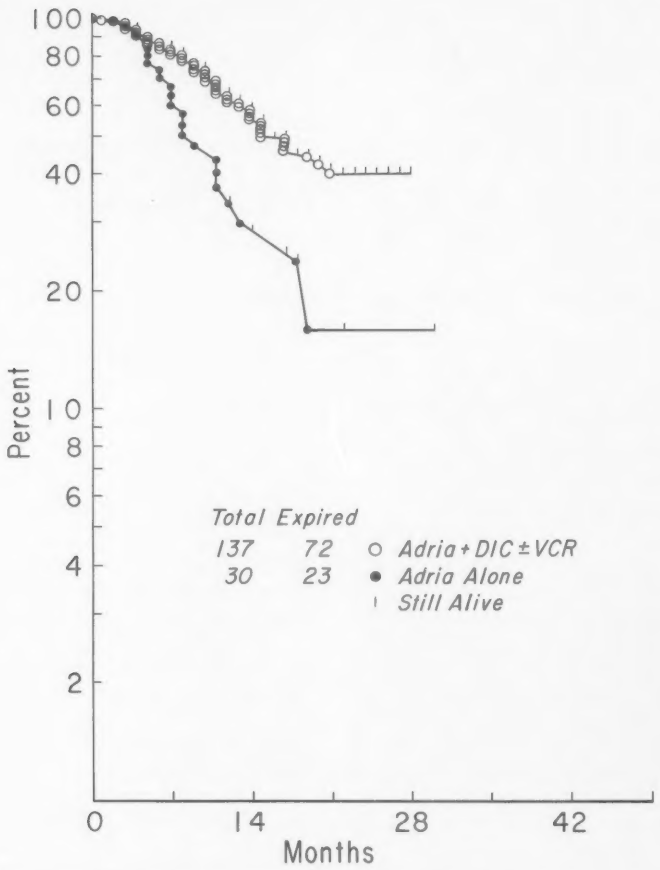

FIGURE 2.—Survival of responding sarcoma patients treated with adriamycin alone (SWOG-442 and SWOG-7206) or adriamycin combined with DTIC with or without vincristine (SWOG-445 and SWOG-7210).

TABLE 2.—Contribution of DTIC to remission duration and survival in patients with sarcomas

	Adriamycin (SWOG)	Adriamycin (BCRC)	Adriamycin + DTIC (SWOG)
No. of patients	97	34	325
Median duration of remission (mos)	4	5.5	5.5+
Median survival of responders (mos)	8	12	15
Median survival of all patients (mos)	6	8	10

tion and survival in the patients treated in the studies described in this paper and the BCRC pilot study has yielded convincing evidence of the superiority of the results obtained when DTIC is used in combination with adriamycin (table 2).

DTIC adds a considerable amount of nausea and vomiting to the toxicity of adriamycin in most patients, especially on the first day of chemotherapy. Patients tend to develop tolerance to this side effect, however, so that less and less nausea occurs on subsequent days of chemotherapy. Other side effects occasionally attributable to this agent were a rare flu-like syndrome, some cumulation of myelosuppressive toxicity, and thrombocytopenia.

CONCLUSIONS

DTIC is an agent with at best modest activity in the therapy of patients with disseminated sarcomas. It is most active against Kaposi's sarcoma and leiomyosarcoma. In combination with adriamycin, it causes some increase in the response rate compared with that seen with adriamycin alone. More importantly, however, DTIC prolongs remission duration and survival and is therefore indicated as part of the first-line treatment in such patients.

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Combination Chemotherapy With DTIC (NSC-45388) in Advanced Malignant Melanoma, Soft Tissue Sarcomas, and Hodgkin's Disease

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SUMMARY

The results of three controlled studies with multiple-drug regimens which included DTIC are reported for advanced melanoma, soft tissue sarcomas, and Hodgkin's disease. In malignant melanoma, no statistical difference was observed between (*a*) DTIC, BCNU, and vincristine, and (*b*) DTIC, BCNU, and actinomycin D in terms of response rate, median duration of response, and survival. In sarcomas, the combination of adriamycin and DTIC produced a higher incidence of complete plus partial responders when compared to cyclophosphamide, vincristine, and methotrexate. However, no significant difference was seen in the median duration of response or survival. In Hodgkin's disease, a new four-drug combination including adriamycin, bleomycin, vinblastine, and DTIC (ABVD) produced an incidence of complete remissions and a median duration of response comparable to that achieved with mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine (MOPP). Preliminary data indicate that ABVD is not cross resistant to MOPP. It is concluded that DTIC can be successfully and safely employed in combination with conventional agents in susceptible neoplastic diseases.

[Cancer Treat Rep 60:205-211, 1976]

We have used DTIC almost exclusively in combination with other anticancer agents in the treatment of advanced malignant melanoma, soft tissue sarcomas, and Hodgkin's disease. This paper reports the results observed in three controlled studies designed during the past few years.

MALIGNANT MELANOMA

In 1971, the World Health Organization's (WHO) Collaborating Centers for Evaluation of Methods of Diagnosis and Treatment of Melanoma designed a prospective controlled study for metastatic disease in order to compare four three-drug combinations which all included DTIC. The detailed results of that study were reported separately (1). Our report will analyze only the experience we have achieved with two three-drug combinations.

Table 1 presents an outline of the treatment schedules and the characteristics of evaluable patients. After physical, radiologic, and radioisotopic examination, patients with a performance status > 40 and a life expectancy > 4 weeks were randomly allocated to either therapy A or therapy B. In the absence of severe myelosuppression, each cycle was repeated on Day 29. In the presence of moderate hematosuppression (decrease in leukocyte count from 3999 to 2000 cells/mm^3 and/or platelet count from 129,000 to 80,000 platelets/mm^3), the dose of each drug was decreased by 50%. Blood counts were usually determined only on Day 1 and Day 15 of each cycle. Treatment was continued until either progression or relapse occurred after an initial response. In patients achieving CR, a total of 12 cycles were administered before treatment was discontinued. The same combination was restarted in the presence of relapse. Practically all patients who were treated were ambulatory. A patient was considered evaluable only if treatment was administered for a minimum of two cycles. Patients were considered not evaluable if they had received only one cycle and then were lost to followup without any evidence of response. Only nine (15%) of 59 patients who were given therapy A and eight (20%) of 40 patients given therapy B had been previously treated with single-agent chemotherapy (with drugs not employed in the combination), or with immunotherapy (bacillus Calmette-Guērin [BCG]), or both.

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TABLE 1.—Treatment schedules and characteristics of evaluable patients with malignant melanoma

Iv treatment	Dose (mg/m^2)/ schedule	No. of evaluable patients* (sex)	No. of patients with prior therapy with— Single-agent chemotherapy	BCG
Therapy A		27 (M), 32 (F)	6	3
DTIC	100/Days 1-5			
BCNU	100/Day 1			
Vincristine	1.4/Days 1 and 15			
Therapy B		17 (M), 23 (F)	4	4
DTIC	100/Days 1-5			
BCNU	100/Day 1			
Actinomycin D	0.05 mg/kg/Days 1 and 15			

*Mean age, 46 yrs.

The comparative evaluation of response is reported in table 2. There is no significant difference with respect to the response level or the duration of response between the two treatment groups. At present, three patients in each treatment group are still in CR for a median of 8 months after discontinuation of therapy. The incidence of CR and PR was not significantly affected by sex. The comparison of response in relation to the site of metastases is shown in table 3. In both groups a higher incidence of CR plus PR was observed in patients with metastatic disease apparently limited to soft tissues compared to those who also had visceral involvement. However, the difference in the response rate between soft tissue and visceral

TABLE 2.—Comparison of response in malignant melanoma

Response type	No. of patients (%) receiving— Therapy A*	Therapy B*
None	31(53)	18(45)
Objective improvement	12(20)	7(18)
PR	9(15)	8(20)
CR	7(12)	7(18)
CR +PR	16(27)	15(38)
Total No. of responses	28(48)	22(55)
Median duration of CR + PR in mos (range)	6+(2–33)	11+(1–34+)

*See table 1 for definitions of therapy schemes.

TABLE 3.—Response related to pattern of metastases in malignant melanoma

Site of metastases	Therapy A* No. of patients treated	Therapy A* CR + PR (%)	Therapy B* No. of patients treated	Therapy B* CR + PR (%)
Skin	9	22	12	75
Lymph nodes	7	42	1	0
Skin + lymph nodes	10	40	5	40
Viscera	15	20	5	20
Viscera + soft tissues	18	22	17	17
All soft tissues	26	35	18	61
All viscera ± soft tissues	33	21	22	18

*See table 1 for definitions of therapy schemes.

TABLE 4.—Comparison of median survival of evaluable patients from beginning of treatment and response in malignant melanoma

Response type	Median survival time in mos (range) of patients receiving— Therapy A*	Therapy B*
None	6 (1.5-29+)	4
Objective improvement	7 (3-19)	11 (5-15)
PR	8 (3-15)	16+(4-34+)
CR	31+(9-42)	25+(9-31+)
CR + PR	11+(3-42)	23+(4-34+)
Total No. of responses	11+(3-42)	14+(4-34+)

*See table 1 for definitions of therapy schemes.

TABLE 5.—Toxic manifestations of patients with malignant melanoma

Toxic effects	% of patients receiving— Therapy A* (59 patients)	Therapy B* (40 patients)
Hematosuppression	50	68
Leukopenia†	31	53
Thrombocytopenia‡	26	18
Anemia§	15	36
Loss of hair	24	58
Oral mucositis	4	23
Neurotoxicity	37	0
None	9	0

*See table 1 for definitions of therapy schemes.
† Wbc <4000 cells/mm³.
‡ Platelet count <130,000 platelets/mm³.
§ Hemoglobin fall <3 g/100 ml below initial values.

disease was statistically significant only in the group treated with therapy B ($P < 0.01$).

Table 4 presents the survival data related to response type. It appears evident that responders, and particularly those who achieved CR, lived longer than nonresponders. There is no statistical difference, however, between the two groups at each level of response. Patients with brain metastases showed a poor survival rate. Those who had disease that also involved the central nervous system before combination chemotherapy was started had a median survival time of 4.6 months (therapy A) and 1.7 months (therapy B). Those who developed brain metastases during treatment survived 1 and 1.4 months, respectively.

Table 5 reports the toxic manifestations that occurred during therapy. A burning sensation along the vein selected for injection was present in most patients during the administration of DTIC. Various degrees of nausea and vomiting occurred in more than half of the patients after the injection of this drug. These side effects were more prominent when DTIC was combined with actinomycin D (therapy B). Actinomycin D was also responsible for the higher incidence of leukopenia, anemia, loss of hair, and stomatitis when compared to therapy A. However, all side effects were reversible and no drug-related deaths were observed.

SOFT TISSUE SARCOMAS

After the initial favorable results reported by Gottlieb et al (2) in soft tissue and bony sarcomas with the combination of full doses of adriamycin and DTIC, a controlled study was started in our Institute to test the therapeutic effects and the absence of cross resistance of two independent combinations in patients with advanced soft tissue sarcomas.

The extent of metastatic disease was determined through physical, radiologic, and radioisotopic examinations. The performance status was > 40 in all patients, and the life expectancy was > 4 weeks. Most of the patients received their treatment in the clinic. Table 6 outlines the treatment schemes. After random allocation treatment was continued in both groups until either progression or relapse after an initial response was evident. At this point, crossover was carried out automatically in three patients receiving therapy A (DTIC plus adriamycin) when the total dose of adriamycin was approaching 550-600 mg/m². Upon further progression after secondary treatment, actinomycin D was administered (1.5-2 mg/m²/week). A dose-reduction schedule similar to that described for malignant melanoma was adopted in the presence of hematosuppression. Ambulatory patients had blood cell counts determined on the same day of drug administration. Patients were considered evaluable if they received a minimum of two cycles of either therapy A or therapy B (cyclophosphamide, vincristine, and methotrexate).

TABLE 6.—Outline of treatments in soft tissue sarcoma

Iv treatment	Dose and schedule	Rest period
Therapy A		
Adriamycin	60 mg/m², Day 1	Days 6-21
DTIC	250 mg/m², Days 1-5	
Therapy B		
Cyclophosphamide	1 g/m², Day 1	
Vincristine	1.4 mg/m², Days 1 and 8	Days 6-21
Methotrexate	20 mg/m², Days 1 and 8	

A comparison of response to therapy A and therapy B is shown in table 7. It appears evident that the combination of therapy A was superior in terms of CR plus PR compared to the triple combination of therapy B. However, this difference was not reflected in the median duration of response. The median survival for complete and partial responders was 10+ months for therapy A compared to 9+ months for therapy B. In nonresponders these findings were 6 and 5.5 months, respectively. The response related to the histologic subgroups is reported in table 8. The limited number of evaluable patients in each treatment group prevents an adequate comparison. Nevertheless, it is worth noting that the incidence of CR plus PR was superior in the adriamycin-DTIC group compared to the triple combination in undifferentiated sarcoma, leiomyosarcoma, liposarcoma, rhabdomyosarcoma, and neurosarcoma. No difference was observed in fibrosarcoma. After crossover, therapy A was given to a group of 17 patients. Secondary treatment induced only one PR for 5+ months in a patient with rhabdomyosarcoma and one objective improvement for 1+ month in a patient with fibrosarcoma. Secondary therapy with the triple combination induced an objective improvement in one (synovial cell sarcoma) of seven patients who relapsed from therapy A. Not enough data are presently available about the therapeutic effect of actinomycin D.

Table 9 presents a comparative evaluation of toxic effects. The observations made for DTIC in malignant melanoma are also valid for soft tissue sarcomas. Aside from the specific toxic effects related to vincristine (paresthesias) and cyclophosphamide (cystitis), thrombocytopenia was more often encountered after therapy A, as well as oral mucositis and loss of hair. These side effects were primarily, if not exclusively, secondary to adriamycin. No patient showed cardiomyopathy; no drug-related deaths were observed. The percent of optimal dose administered was as follows: adriamycin, 92%; DTIC, 91%; cyclophosphamide, 85%; methotrexate, 81%; vincristine, 82%.

HODGKIN'S DISEASE

As reported previously (3-5), all mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine (MOPP)-modified regimens failed to yield better results in Hodgkin's disease than MOPP in terms of incidence and median duration of CR. For this reason, a new combination was designed in our Institute in order to develop an effective non-cross-resistant regimen which could be employed either in MOPP failures or in sequence with MOPP. This

TABLE 7.—Response to primary treatment of soft tissue sarcomas

	Therapy A*	Therapy B*
No. of patients entered	31	32
No. of patients evaluable	21	28
CR	19%	11%
PR	38%	4%
Objective improvements	29%	28%
CR + PR	57%	15%
Total responses	86%	43%
Median duration in mos (range) of—		
CR	7.5 (3-10+)	10 (2+-13+)
PR	3 (1+-9+)	2
CR + PR	5 (1+-10+)	6 (2-13+)

*See table 6 for definitions of therapy schemes.

TABLE 8.—CR + PR related to histologic subgroups in soft tissue sarcomas

	No. of responses/total No. of evaluable patients receiving—	
Histologic type	Therapy A*	Therapy B*
Undifferentiated	5/5	0/3
Fibrosarcoma	1/6	1/7
Leiomyosarcoma	1/1	0/3
Liposarcoma	2/2	0/5
Rhabdomyosarcoma	1/2	1/5
Synovial cell sarcoma	0/2	—
Angiosarcoma	—	1/2
Neurosarcoma	2/3	0/2

*See table 6 for definitions of therapy schemes.

TABLE 9.—Toxic manifestations after primary therapy for soft tissue sarcomas

	% of patients receiving—	
Toxic effects	Therapy A*	Therapy B*
Leukopenia†	43	61
Thrombocytopenia‡	48	25
Anemia§	33	25
Oral mucositis	29	18
Loss of hair	95	75
Paresthesias	—	46
Cystitis	—	18
None	0	14

*See table 6 for definitions of therapy schemes.
†Wbc < 4000 cells/mm^3.
‡ Platelet count < 150,000 platelets/mm^3.
§ Hemoglobin fall > 3 g/100 ml below initial values.

cyclic four-drug combination includes adriamycin, bleomycin, vinblastine, and DTIC (ABVD), none of which are present in the MOPP regimen. All drugs selected for this four-drug combination are known to be effective in Hodgkin's disease. In particular, besides the well-established effect of adriamycin (6), bleomycin (7), and vinblastine (8), DTIC was recently reported by Frei et al (9) to produce CRs or PRs in 56% of patients with Hodgkin's disease who were resistant to many standard agents. Frei's results were confirmed by our group in a small series of patients (CR or PR in five of six patients [3]).

The details of the MOPP versus ABVD study have been described in previous publications (3-5). In short, patients with pathologic stages IIB, IIIB, III_S (A and B), and IV were randomly allocated to six cycles of either combination (induction phase). No patients had had previous chemotherapy. Patients achieving either a CR or a PR of > 75% (but < 100%) regression were subsequently given irradiation (consolidation phase) with 3000-3500 rads.

At the end of radiotherapy no further treatment was given to complete responders who initially had only nodal extent. On the other hand, those patients classified as having stage IV disease who were in CR after low-dose (2000-3000 rads) irradiation had been delivered to sites of major pretreatment involvement were given six more cycles of either MOPP or ABVD every 2 months (maintenance phase).

TABLE 10.—Outline of 1 cycle of ABVD therapy

Iv drug	Dose (mg/m^2)	Treatment on Day— 1	2	3	4	5	14	15	28
Adriamycin	25	×					×		
Bleomycin	10	×					×	Rest	
Vinblastine	6	×					×	period	
DTIC	150	×	×	×	×	×			

Table 10 presents the original observations with ABVD when DTIC was administered during the first 5 days of each cycle. In September 1974, to limit a major patient complaint of nausea and vomiting after each drug injection, the dose of DTIC was also concentrated (375 mg/m^2) on Days 1 and 14. A dose-attenuation schedule was used in the presence of myelosuppression, severe stomatitis, and severe neurotoxicity (3). Almost all patients were managed and treated in the outpatient clinic, and for most of them, the dose adjustments were carried out on the basis of blood cell counts determined before starting each course. Patients failing to respond to the induction phase or patients relapsing after a CR were crossed over.

The comparative therapeutic results between MOPP and ABVD given as primary treatment are presented in table 11. No difference was observed between the two combinations in terms of CRs (74% versus 77%). It is worth recalling that in patients with stage IV disease a second biopsy of accessible extralymphatic sites of known pretreatment involvement (eg, liver, bone marrow) had to be interpreted as negative to consider a patient in a state of CR. The therapeutic comparison in relation to histologic subtypes and prior irradiation also failed to reveal a significant difference between the two treatment groups, indicating that the percent of complete responders was not influenced by prior radiotherapy. The preliminary analysis of median duration of CR provides a further indication that ABVD appears to be as effective as MOPP. The results of secondary therapy after crossover showed that a CR or PR was observed in four of five patients given MOPP and in four of six patients treated with ABVD.

In terms of myelosuppression there was not an appreciable difference between MOPP and ABVD (table 12). The latter combination induced a higher incidence of marked loss of hair when compared to MOPP. In less than half the patients treated with ABVD mild skin changes were present as hyperpigmented stria secondary to cutaneous deposits of

TABLE 11.—Comparative response between MOPP and ABVD as primary treatment in Hodgkin's disease

Disease stage	MOPP No. of patients treated	MOPP CR (%)	MOPP Median duration in mos (range)	ABVD No. of patients treated	ABVD CR (%)	ABVD Median duration in mos (range)
IIB	3	100	4 (2-7)	3	100	2.5 (1-7)
IIIB-III_S	16	75	7 (3-15)	18	72	7 (2-12)
IV	16	69	10 (4-15)	14	79	8 (2-16)
Totals	35	74	7+(2-15)	35	77	7+(1-16)

TABLE 12.—Toxic manifestations of patients with Hodgkin's disease

Toxic effects	% of patients receiving— MOPP (35 patients)	ABVD (35 patients)
Leukopenia*	60	71
Thrombocytopenia†	25	11
Oral mucositis	—	3
Loss of hair	31	71
Paresthesias	74	3
Skin changes	—	46
Pulmonary fibrosis	—	—
Cardiomyopathy	—	—

*Wbc < 4000 cells/mm³.
†Platelet count < 150,000 platelets/mm³.

bleomycin. The incidence of stomatitis was almost nil. No patients showed signs of pulmonary fibrosis or cardiomyopathy. All stage IV patients received a total dose of 250 mg/m² of adriamycin. The percent of optimal dose for each drug in the ABVD regimen was as follows: adriamycin and vinblastine, 87%; DTIC and bleomycin, 96%. In the MOPP combination the findings were: mechlorethamine and procarbazine, 89%; vincristine, 88%; prednisone, 100%.

COMMENT

The results of our studies show that DTIC can be successfully and safely employed in combination with conventional agents in the treatment of malignant melanoma, soft tissue sarcomas, and Hodgkin's disease. Since DTIC produces only a moderate degree of myelosuppression in most patients, its use appears particularly indicated in combination chemotherapy where its administration is compatible with optimal or near optimal doses of myelosuppressive drugs.

The treatment of advanced malignant melanoma appears difficult with presently available agents. Almost all studies have come to the conclusion that DTIC is the drug of choice (10). Unfortunately, in all studies but one (11) combination chemotherapy with DTIC failed to produce a significantly higher incidence of CR plus PR when randomly or retrospectively compared to series treated with DTIC alone. Our results as well as those of the WHO Collaborating Centres for Evaluation of Methods of Diagnosis and Treatment of Melanoma (1) are consistent with these findings. Thus, the possibility of increasing the incidence of responders as well as the duration of response and survival in advanced disease appears, at the present moment, to be influenced in a number of patients by the combined use of chemotherapy plus immunotherapy (12). A more strategically adequate treatment of melanoma consists of prolonged adjuvant immunotherapy (BCG), with or without DTIC (13), in high-risk patients with operable disease.

Recent data on soft tissue and bone sarcomas indicate that an increased response rate as well as an improved survival rate (14,15) were obtained when adriamycin was combined with other agents including DTIC. Our results also show that the combination of adriamycin plus DTIC is superior to a triple combination of conventional agents, and this is probably due to the fact that when combined with adriamycin, DTIC is synergistic in man as well as in experimental systems (16). This combination with or without cyclophosphamide and vincristine is now being studied as surgical adjuvant therapy for sarcomas in adults and children.

In advanced Hodgkin's disease the need for an effective multiple-drug regimen non-cross-resistant to MOPP has been recently stressed (3,4). Preliminary results indicate that ABVD could be considered a combination which meets the above-mentioned requirements. The role of DTIC in such a combination could be important not only because this drug is effective in refractory Hodgkin's disease, but also because of its synergistic activity when combined with adriamycin.

LIST OF COMPOUNDS

ABVD: adriamycin, bleomycin, vinblastine, and DTIC
Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
MOPP: mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1, 4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Clinical Studies With DTIC (NSC-45388) in Various Malignancies[1]

Milan Slavik[2,*]

The role of DTIC in malignant melanoma, soft tissue sarcoma, and malignant lymphoma has been discussed in other papers presented at this meeting. My presentation is limited to the clinical studies of this drug in other malignancies (table 1). The results from a clinical study in children with solid tumors indicate DTIC activity in neuroblastoma and rhabdomyosarcoma (1). However, the same group has also found this drug ineffective in acute lymphoblastic leukemia in children (2). In adults the drug has not revealed any activity in gastrointestinal, genitourinary, or gynecologic malignancies.[3] Detailed information on 84 patients with large bowel cancer from four different studies is given in table 1.

In spite of the lack of significant activity of DTIC in large bowel cancer, this drug has been used in combination with 5-fluorouracil (5-FU), vincristine, and BCNU and in a controlled randomized study by Falkson et al (5) (fig 1) which compared the combination to 5-FU alone.[4] The results from this study are shown in table 1. Interestingly enough, these results motivated Moertel et al (6) to explore in a similar manner the three-drug combination of methyl-CCNU,[5] vincristine, and 5-FU. Because of their past experience with DTIC they did not include this drug in the combination, but they achieved the same high response rates. DTIC has also shown no significant activity against lung cancer; the cumulative data filed with the Investigational Drug Branch show a 10% overall response rate (14 responders among 137 patients).

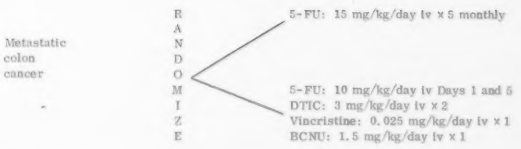
Metastatic colon cancer
RANDOM
5-FU: 15 mg/kg/day iv x 5 monthly
5-FU: 10 mg/kg/day iv Days 1 and 5
DTIC: 3 mg/kg/day iv x 2
Vincristine: 0.025 mg/kg/day iv x 1
BCNU: 1.5 mg/kg/day iv x 1

FIGURE 1.—Treatment scheme of Falkson et al (5) for colon cancer.

DTIC has not revealed any activity as a single agent in breast cancer and a summary of the three studies in this disease is given in table 1. DTIC has established activity in malignant melanoma and is active in soft tissue sarcomas, malignant lymphomas, and two childhood solid tumors, neuroblastoma and rhabdomyosarcoma. Although it has not revealed significant activity in the other major solid tumors in adults, it should be noted that for most of these tumors this drug has not yet been adequately tested in disease-oriented phase II studies.

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[1]DTIC: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

[2]Investigational Drug Branch, Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[3]Investigational Drug Branch, CTEP, DCT, NCI. Cumulative data.

[4]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

[5]Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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TABLE 1.—Activity of DTIC in various malignancies

Study	Dosage	Tumor type	No. of patients— Evaluable	No. of patients— Responding	Response rate (%)
Solid tumors in children (Finklestein et al, ref 1)	200-470 mg/m²/day × 5 q22d	Neuroblastoma	18(13)*	3	16
		Rhabdomyosarcoma	11(7)	1	9
		Wilms' tumor	4(3)	0	—
		Ewing's sarcoma	3(1)	0	—
		Miscellaneous	10(6)	0	—
		Total	46(30)	4	9(13)
Leukemia (Children's Cancer Study Group A, Finklestein et al, ref 2)	150-250 mg/m²/day × 5, first course, followed 3-9 days later by 150-500 mg/m²/day × 5; subsequent doses adjusted individually	Acute lymphocytic leukemia	20	0	0
Solid tumors in adults	—	*Gastrointestinal*			
		Esophagus	3	0	0
		Stomach	10	2	20
		Pancreas	10	0	0
		Hepatoma	6	0	0
		Bile duct	7	1	14
		Large bowel	84	7	8
		Genitourinary			
		Kidney	29	1	3
		Bladder	7	0	0
		Prostate	6	0	0
		Testis	4	0	0
		Gynecologix			
		Ovary	9	0	0
		Uterus	5	0	0
		Cervix	12	3	25
Colorectal cancer					
Southwest Cancer Chemotherapy Study Group, No. 434	250-375 mg/m²/day × 5 q3wks	—	19	1 PR	4
Luce et al (ref 3)	250 mg/m²/day × 5 q3wks	—	4	0	0
Moertel et al (ref 4)	200-600 mg/m²/day × 5 q35d	—	19	1 PR	4
Central Oncology Group, No. 530	4.5 mg/kg/day × 10 q28d	—	42	5 PR	12
Total			84	7 PR	8
Colon cancer (Falkson et al, ref 5)					
5-FU alone	—	—	24	2 CR, 4 PR	25†
5-FU, DTIC, vincristine, BCNU	—	—	28	4 CR, 8 PR	43‡
Breast cancer					
Central Oncology Group, No. 530	4.5 mg/kg/day × 10 q28d	—	37	5 PR	14
Southwest Cancer Chemotherapy Study Group, No. 434	250-375 mg/m²/day × 5 q21d	—	10	0	0
Luce et al (ref 3)	250 mg/m²/day × 5 q21d	—	4	0	0
Total			51	5	10

*Nos. in parentheses are patients who received 2 or more courses.
†Median duration of response = 3.3 mos.
‡Median duration of response = 4.5 mos.

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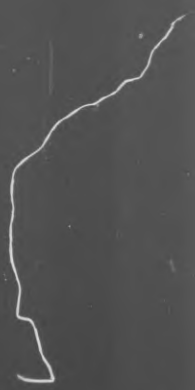
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